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NATIONAL SHELLFISH SANITATION PROGRAM: DEPURATION PLANT DESIGN

Santo A. Furfari

Public Health Service
Washington, D. C.

1966

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NATIONAL SHELLFISH SANITATION PROGRAM

DEPURATION PLANT DESIGN

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U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Division of Environmental Engineering and Food Protection
Washington, D.C. 20201
1966

FOREWORD

The National Shellfish Sanitation Program was established in 1925 through joint action of interested State and Federal health and conservation agencies and the shellfish industry. The primary goal of the program is to provide assurance to consumers that shellfish, oysters, clams, and mussels are a safe food and thereby to retain the public confidence in the industry. As such the National Program is concerned with all factors in the growing, harvesting, processing, and distribution of shellfish which have a significant bearing on the attainment of this goal. Depuration is one such factor.

Depuration is a process in which shellfish are held in a clean water environment for a time sufficient to permit them to free themselves of transient pollutional microorganisms through normal biological processes. Depuration is therefore the application of normal biological principles to the better attainment of a public health goal. While the process has been widely used in Europe, where it was developed many years ago, it has not been adopted by the United States industry nor has its use been required by public health officials in the United States.

The informational and technical guidelines presented in this report are based on limited use of commercial depuration in the United States, on biological research not necessarily directed toward the process, on depuration-oriented research projects undertaken by State and Federal agencies in the United States and Canada, and on an analysis of European experience with the process. The guidelines are intended to assist the shellfish industry in the initial design, construction, and operation of shellfish depuration plants, and to guide State agencies in the establishment of meaningful administrative controls. It is anticipated that these guidelines will be revised as additional commercial experience is accumulated, and as the results of additional research projects become available.

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TABLE OF CONTENTS

	Page No.
FOREWORD	i
DEFINITIONS	iv
INTRODUCTION	1
OPERATIONAL GUIDELINES	3
Typical Depuration Plants	3
Location of Depuration Plants	3
Sea Water Quality	7
Treatment of Sea Water	7
Special Treatment	12
Ultraviolet Light	12
Filtration	17
Time for Depuration and Expected Bacterial Results	17
Controlled Storage	18
Laboratory Control and Sampling Procedures	20
DESIGN CRITERIA	23
Practices	
Sea Water Flow Rates	23
Tank Washing	23
Shellfish Washing and Culling	24
Waste Handling	27
Construction	
General	28
Lighting	28
Materials	28
Pipes and Piping Systems	29
Depuration Tanks and Linings	31
Pumps	32
Baskets	32
PLANT SIZE AND ESTIMATED DESIGNING FACTORS	34
APPENDICES	39
BIBLIOGRAPHY	111

LIST OF FIGURES

Figure No.	Description	Page No.
1	Depuration Plant Layouts	4
2	Ultraviolet Light Treatment Unit	13
3	Flow Limitations on U.V. Treatment Unit.	14
4	Typical Log Sheet for U.V. Tubes	16
5	Washing and Culling Machine	26

LIST OF TABLES

Table No.	Description	Page No.
1	Quality of Source of Sea Water	6
2	Quality of Depuration Sea Water	8
3	Time for Depuration and Bacterial Quality After Depuration	19
4	Pipe for Depuration Plants	30
5	Tank Sizing Factors	31
6	Designing Factors for Depuration Plants.	35

DEFINITIONS & TERMINOLOGY

ABSORPTION COEFFICIENT - Absorption coefficient describes a property of a translucent medium (sea water, e.g.) concerning the light transmittance capacity of the medium. The unit is usually per centimeter.

CLOSED SYSTEM, RECIRCULATING SYSTEM - These terms are used interchangeably to describe a sea water system in a depuration plant in which the water, after contacting the shellfish, is returned to certain treatment units and re-used repeatedly by the shellfish (as opposed to OPEN SYSTEM, below).

COLIFORM BACTERIA - The coliform group of bacteria includes all of the aerobic and facultative anaerobic, Gram-negative, non-spore-forming bacilli which ferment lactose with gas formation within 48 hours at 35 degrees C. Bacteria of this group which will produce gas from E.C. medium within 24 hours at 44.5 degrees C in a water bath will be referred to as fecal or E.C. positive coliforms.

CULLING - Culling is the process of separating dead or broken shellfish from the live, intact shellfish.

DEPURATION - Depuration is the process in which shellfish of doubtful hygienic quality are made acceptable for human consumption and still retain their living state. In the usual sense, depuration means self-purification by natural biological processes.

DEPURATION PLANT - A depuration plant consists of equipment suitably arranged to carry out the depuration process.

LABORATORY CONTROL - Laboratory control is the term used to describe the operational aspect of depuration plants in which the quality of shellfish and sea water is measured, controlled and recorded.

MANTLE FLUID - The mantle fluid of shellfish refers to the liquid which is contained in the mantle chamber.

OPEN, FLOW-THROUGH, SINGLE-PASS, SYSTEMS - These terms are used interchangeably to describe a sea water system in a depuration plant in which sea water is pumped into the plant and its tank and immediately goes to waste, after contact with shellfish.

OYSTER - Unless otherwise specified this term refers to the family of oysters, Ostreidae.

PESTICIDES - This term refers to any chemical substance, either organic or inorganic which is used to control insects, weeds, or small animals.

QUAHAUG, HARD CLAM - Unless otherwise specified this term refers to the species or subspecies of Mercenaria (Venus) mercenaria.

SHELLFISH - The shellfish of concern in this report include edible species which may be subject to depuration, such as:

Locale	Common Name	Scientific Name
Atlantic Coast	Soft-Shell Clam, Long Necks	Mya arenaria
	Northern Quahaug, Hard Clam	Mercenaria mercenaria
	Ocean Quahaug	Cyprina Islandica
	Sea Clam	Spisula solidissima
	Blue Mussel	Mytilus edulis
	Eastern Oyster (also Gulf Coast)	Crassostrea virginica
Gulf Coast	Southern Quahaug	Mercenaria campechiensis
	Horse Oyster (Crested Oyster)	Ostrea equestris
West Coast	Olympia, Native Oyster	Ostrea lurida
	Pacific, Japanese Oyster	Crassostrea gigas
	Manila Clams	Tapes japonica
	Little Neck	Tapes staminea (or Paphia staminea)
	Native Little Neck	Protothaca staminea
	Butter Clam	Saxidomus nuttali and/or giganteus
	Razor Clam	Siliqua patula

STORAGE, DRY - The storage of shellstock out of water.

TOXIC - This term is used for both man and shellfish. A substance toxic to shellfish prevents high activity of the shellfish and therefore prevents satisfactory depuration. A substance toxic to man makes him ill.

TURBIDITY - Turbidity refers to the particles in water which reduce the light transmittance as measured by a Jackson Candle Turbidimeter. Units are usually given as Turbidity Units or sometimes as J.T.U.

ULTRAVIOLET LIGHT - The ultraviolet light referred to in this report includes the bactericidal wave length of light of 2,537 Angstrom Units. The intensity is usually given a micro-watts/cm^2. This intensity multiplied by a time factor is called a dose.

ZERO HOUR - The time at which shellfish begin to depurate is referred to as the zero hour. This usually can be taken as the time at which a tank becomes full with depuration water.

DEPURATION PLANT DESIGN

INTRODUCTION

The technical guidelines contained in this report are based on the available information derived from research, pilot plant studies and existing commercial plants. These guidelines are directed mainly toward defining the environment of the shellfish which is favorable to the depuration process. From these guidelines certain plant design criteria have been established to aid in the construction of plants and estimating their cost.

Depending on the States' requirements of designation of areas for the harvesting of shellfish to be depurated, the quality of the untreated shellfish may range from those shellfish harvested from excellent approved waters to those harvested from restricted waters. This range describes shellfish in which there is a very low probability of contact with harmful micro-organisms to those in which there may be some probability of this contact. With the increasing use of all waters for recreation, all shellfish can be suspect since even wastes from small boats may impose a pollution load on an area.

Furthermore, shellfish containing pesticides, paralytic shellfish poison and toxic metals are not amenable to the depuration process described, since the time required for self-purification from these contaminants is comparatively long to that time for microbiological reduction. Chemicals are usually bound in tissue.

The success of the depuration process is in the complete purging of the mantle fluid and the gut contents of the shellfish in an allotted time period. The depuration process is optimized with the attainment of the cleanest shellfish in the shortest period of time. It is necessary, therefore, to present to the shellfish an environment which optimizes their feeding and activity, so that pathogenic micro-organisms of concern to man are eliminated.

The concept of depuration as a process of public health importance is not new. Herdman and Boyce, in 1899, experimented with oysters and S. typhi. Records of the period up to 1921 showed activity in research and experimentation both in the United States and Europe. Wells conducted experiments for the U. S. Public Health Service beginning in 1915.

The employment of the concept in commercial practices is not new either. As is well known, mussels have been purified commercially in Wales, United Kingdom, since 1928. France, Spain and Portugal have been prominent in their use of the process for many years.

The objectives of this report are to present the principles upon which the satisfactory depuration of shellfish depend and to establish guidelines for properly accomplishing a depuration program. The principal factors include the quantity and quality of sea water, time for depuration, construction of the plant, location and laboratory control. Plant operation guidelines are also presented.

The Appendices give (a) detailed discussions and derivations of the guidelines, (b) a synoptic history of depuration research and experience, (c) economics, and (d) quality control.

Administrative aspects of (a) the designation of shellfish growing areas, (b) supervision over harvesting and (c) transportation are not covered here. Since the shellfish must be in a biologically active condition in the depuration process, it is important that handling and transportation from the time of harvest to the time of depuration must not shock the animal into an inactive and/or hibernating state.

OPERATIONAL GUIDELINES

Typical Depuration Plants

The typical depuration plant is comprised of four essential units. They are controlled dry storage for untreated and treated shellfish; washing and culling facilities for untreated and treated shellfish; depuration tanks or basins; and sea water treatment. Laboratory and office space may or may not be necessary depending on the particular situation. For large plants, conveyor belts or overhead traveling cranes and hoists for lifting large loads of shellfish are desirable. Complete separation of treated and untreated shellfish by means of physical barriers or process flow lines is required in the layout of a plant.

Figure 1 presents schematic layouts of depuration plants containing the above features. The governing factor in these layouts is the orderly flow of shellfish, i.e., separation of treated from untreated lots. The two layouts of Figure 1 are essentially the same except that the upper one is highly idealized. The lower folded layout is more realistic in that the controlled storage units are closer together for economy of space.

When the storage temperature and humidity requirements are the same for both treated and untreated shellfish, the controlled storage may be comprised of one unit, but with a non-passable partition separating the treated and untreated lots. In this instance there must be separate, clearly labelled doors to each compartment. This particular setup was built into a commercial soft-clam plant in Phippsburg, Maine.

Location of Depuration Plants

For economic reasons, plants should be located for easy access by vehicle and/or boat. The availability of utility services such as electricity, water and waste disposal is also important. Wastes (solids and liquids) are produced at a plant and must be disposed of properly. The main concern with these wastes is the possible contamination of shellfish either directly or indirectly. The source of the process sea water must not be contaminated by plant wastes.

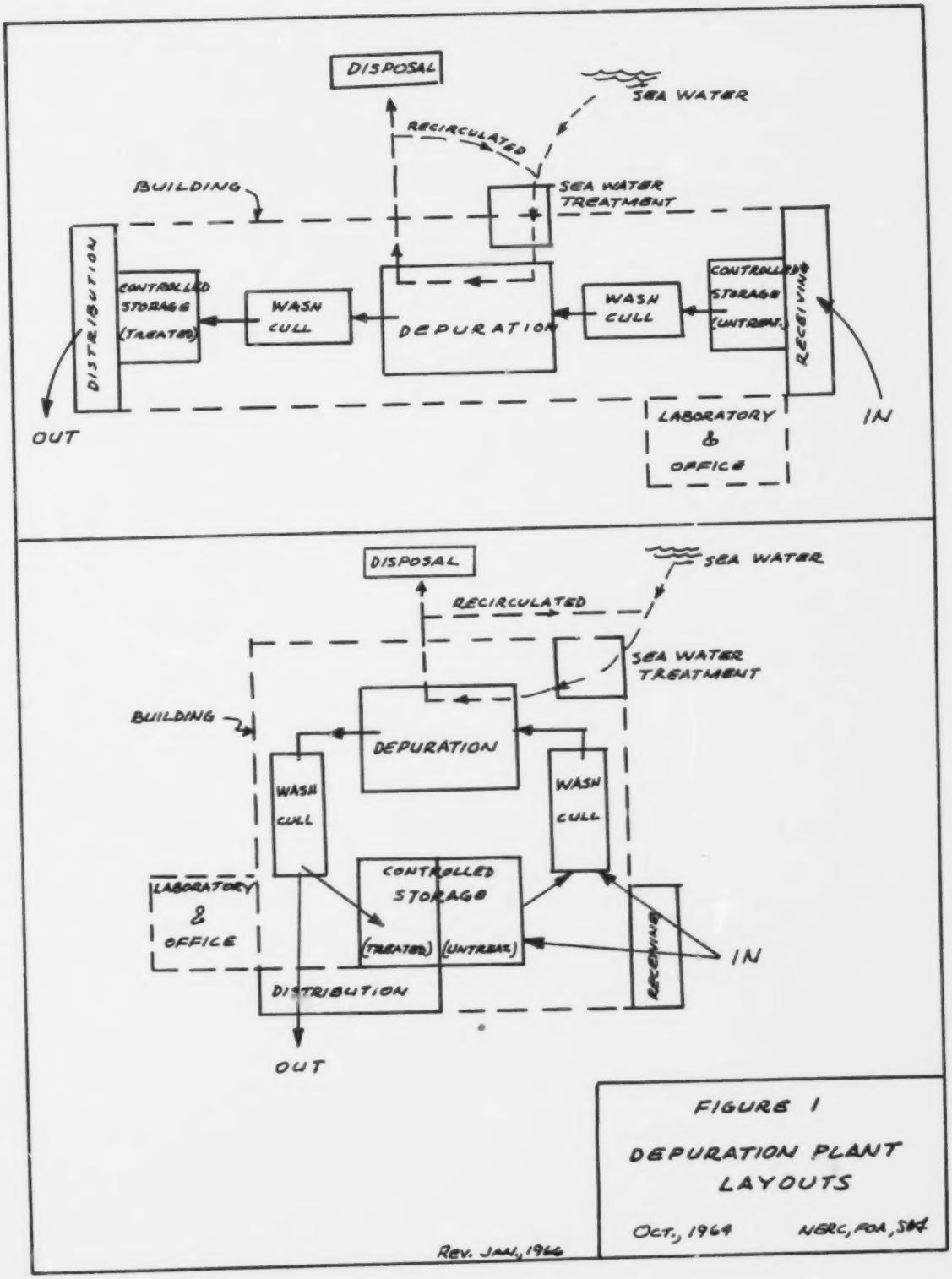


FIGURE 1

DEPURATION PLANT LAYOUTS

OCT., 1964 NERC, FOA, SAF

Rev. JAN., 1966

The location of the plant in relation to the designated harvest areas is important. Transportation time must be kept to a minimum, not only from the viewpoint of bacterial multiplication, but from the aspect of induced stress in the shellfish.

For technical reasons plant location is dependent largely on sea water quality. The presence of the substances listed in Table 1 above the levels described, militates against the use of that water as a source of process sea water unless extraordinary corrective measures are taken.

Water of a lower coliform content is desired, but the requirement given conforms to that given in Part I of the National Shellfish Program Manual. The metals include chromium, iron, lead, mercury, zinc and copper, which may be toxic to shellfish and/or man. If they are toxic to shellfish, the shellfish may remain closed and therefore will not depurate. Practical limits for the organics cited are difficult to set. Sophisticated instruments can detect these substances to extremely low concentrations. The main concern, of course, is their concentration in the shellfish after the shellfish have been exposed to the water for a few days. Many substances are accumulated to cause not only a public health problem, but also a taste, odor and color problem to the consumer.

It is recognized that where depuration plants are most needed, i.e., in the vicinity of pollution, pristine pure sea water is usually unavailable. However, it is highly desirable that depuration plants be located as far (time, distance and dilution) from sources of wastes as possible.

TABLE 1. QUALITY OF SOURCE SEA WATER

Substance	Limits
Coliform - MPN/100ml	Median of samples equal to or less than 700 (Not more than 10% of samples shall exceed 2300 for a 5-tube, 3-dilution test; and 3300 for a 3-tube, 3-dilution test).
Turbidity	20 Jackson Candle Turbidity Units
Metallic Ions and Compounds	Potentially toxic metals shall not be in concentrations above that normally found in sea water.
Organics and Radioisotopes	Pesticides, detergents, dye stuffs, radioisotopes and marine toxins shall not be in concentrations in water above that which can cause concentrations in shellfish which are unacceptable by the regulations of the Food and Drug Administration, DHEW. (In the case of paralytic shellfish poison: 80 micrograms per 100 grams of the edible portion.)

Sea Water Quality

Table 2 presents the essential sea water quality which must be met for presenting an environment suitable to shellfish activity and thereby to successful depuration. It is expected that observance of these limits will prevent shock, stresses and inactivity of the shellfish or, minimize the time required for the shellfish to acclimatize to its arcificial environment. These values can be used for closed or open sea water systems.

Treatment of the sea water to meet these standards may or may not be necessary depending on the sanitary quality of the source.

A discussion on the derivation of these values is presented in the Appendix.

Treatment of Sea Water

The following paragraphs elaborate on the sea water quality items in Table 2 by giving reasons for their significance in terms of shellfish activity and consequently public health. Following each of the particular brief discussions are descriptions of methods of treatment which will correct the deficiencies in quality.

Temperature markedly affects the "activity" of shellfish as is explained in Appendix I-A. Of critical importance to activity of hard clams and oysters are extremely low temperatures. Low temperatures generally cause hibernation and inactivity of most shellfish. Activity is only partly related to depuration.

Spawning may be induced by placing ripe shellfish into water warmer than they were previously in, or else by suddenly increasing the water temperature. The question remains whether or not spawning interferes with effective depuration. Water into which shellfish have spawned becomes highly turbid. This phenomenon may not only reduce depuration rates, but will definitely affect ultraviolet light treatment of water in closed systems. A given species of shellfish has different spawning temperature requirements not only from other species, but from the same species in different geographical areas of the country.

Sea water temperatures should be carefully monitored and controlled during the process, because of the lack of knowledge of its effect on depuration rates.

TABLE 2. QUALITY OF DEPURATION SEA WATER

Item		Minimum	Maximum
Physical Quality			
Temperature*:	Soft Clam	35°F (2°C)	68°F (20°C)
	Hard Clam	50°F (10°C)	68°F (20°C)
	Oyster	50°F (10°C)	77°F (25°C)
Turbidity*		---	20 Units
Chemical Quality			
pH		7.0	8.4
Salinity*		Within (100±20)% of the harvest area value.	
Oxygen, milligrams/liter		5.0	Saturation at temperature
Metals, Organics, Marine Toxins, etc.		Refer to Table 1.	
Bacteriological			
Coliform MPN/100ml		---	1.0

*Items marked with an asterisk may be subject to geographical and local differences. For example, Gulf Coast oysters may depurate just as effectively at 28°C as at 25°C, and at turbidities up to 100 J.T.U. However, unless the differences are shown experimentally, the stated guidelines should be adhered to.

In northern areas heat exchangers will probably be necessary and should be designed to heat sea water from about 32°F (0°C) to the minimum values given in Table 2. In winter the lower temperatures of Table 2 should be aimed for so as to minimize stress on oysters and hard clams (as well as for economic reasons). Heating of the sea water for soft clam depuration is not usually necessary. In any area where sea water temperatures at depuration plants may markedly exceed the water temperatures of harvest areas cooling of the sea water may be required.

Excessive turbidity in the process sea water is of concern for two main reasons. It can (1) reduce the feeding activity of the shellfish and (2) reduce the penetrating power of ultraviolet light. Also turbidity is commercially undesirable as it may affect the palatability of the shellfish. Furthermore, excessive turbidity causes maintenance problems for pumps, pipe and tanks.

The removal of turbidity in excess of 20 J.T.U. may be accomplished by several methods. A most important consideration is, that by any method, food particles for the shellfish should not be removed. It is not known whether rate of depuration, gut purging, or feeding is dependent on the availability of food. Settling will remove turbidity to a certain extent provided sufficient detention time is realized. Usually two hours may be required. Colloidal substances will not be removed in this time and therefore settling rate experiments should be done to determine if there is a problem. Filtration will usually remove most turbidity but food particles will be removed also. Filtration is discussed further, in a later section. Centrifuging of the sea water may be effective in removing some of the heavy particles without removing the lower weight food, but experimentation is necessary depending on the particular situation.

Shellfish activity is dependent on changes of salinity. Shellfish activity is reduced for a certain length of time when salinity is either increased or decreased to above or below that to which the shellfish is accustomed.

The salinity range requirements may be met by choosing an appropriate plant site. If the plant is to be located in estuaries then consideration must be given to the variation in salinity over a tidal cycle as well as at times of high surface runoff. As an example of the use of the ±20% range given in Table 2, if shellfish are harvested from an area having a salinity of 24 o/oo (parts per thousand - ppt), then the allowable range of salinity of the water at the depuration plant is 24 ppt ± 4.8 ppt (19.2 to 28.8 ppt).

High salinity sea water may have to be diluted with fresh water to meet the requirement. Substances in the fresh water (chlorine, e.g.) of course must not affect adversely shellfish activity. The increasing of salinity by the addition of salts to correct an extremely low salinity sea water is not highly recommended at this time, because of the lack of knowledge of the subject. Wood (1961) recommends an artificial sea water for use with European oysters as follows:

Na Cl	10.688 kg.
Mg SO_4 .$7H_2O$	2.637 kg.
Mg Cl_2 .$6H_2O$	2.041 kg.
Ca Cl_2 .$2H_2O$	0.553 kg.
K Cl	0.269 kg.

(Dissolve in 100 British* gallons of water to produce a salinity of 32 o/oo.) Artificial sea waters can be made from other recipes, but experiments must be conducted to determine the effect on depuration rates of shellfish. Allen, et al, (1950) gives recipes for other artificial sea waters used experimentally.

Dissolved oxygen is an important factor in shellfish activity and is especially important in a recirculating sea water system. In a recirculating system the oxygen in the water is gradually depleted by respiration of the shellfish. A minimum of 5 milligrams per liter is derived in Appendix I-F. A percent saturation value is not used because oxygen saturation values are dependent on temperature and salinity. Super saturation of dissolved oxygen may cause reduced shellfish activity, although this has not been shown experimentally.

*120 U. S. gallons.

An aeration device should be provided if oxygen content during recirculation becomes less than 5 mg/l. Two rather inexpensive means of aeration include bubbling and cascading. The bubbling of air into the depuration system usually requires air pumps, piping and an air diffusing device. Air should not be bubbled directly into depuration tanks because the agitation may disturb the shellfish and also may stir up the feces and thus recontaminate the water. Cascading merely requires the water to free-fall and tumble a distance of at least four feet. Spray aeration may be feasible also. When the source of sea water contains less than 5 mg/l of dissolved oxygen, there may be a source of pollution with a high oxygen demand. If this is the case, this source may be questionable for use in a depuration plant.

Shellfish concentrate some metals and organics in extraordinary ratios of body content to water content. Therefore, the presence of a source of industrial wastes containing toxic metals or organics, or a source of runoff containing pesticides, prohibit the locating of a depuration plant in a given area. Treatments to remove these elements and compounds would be usually expensive and difficult to control.

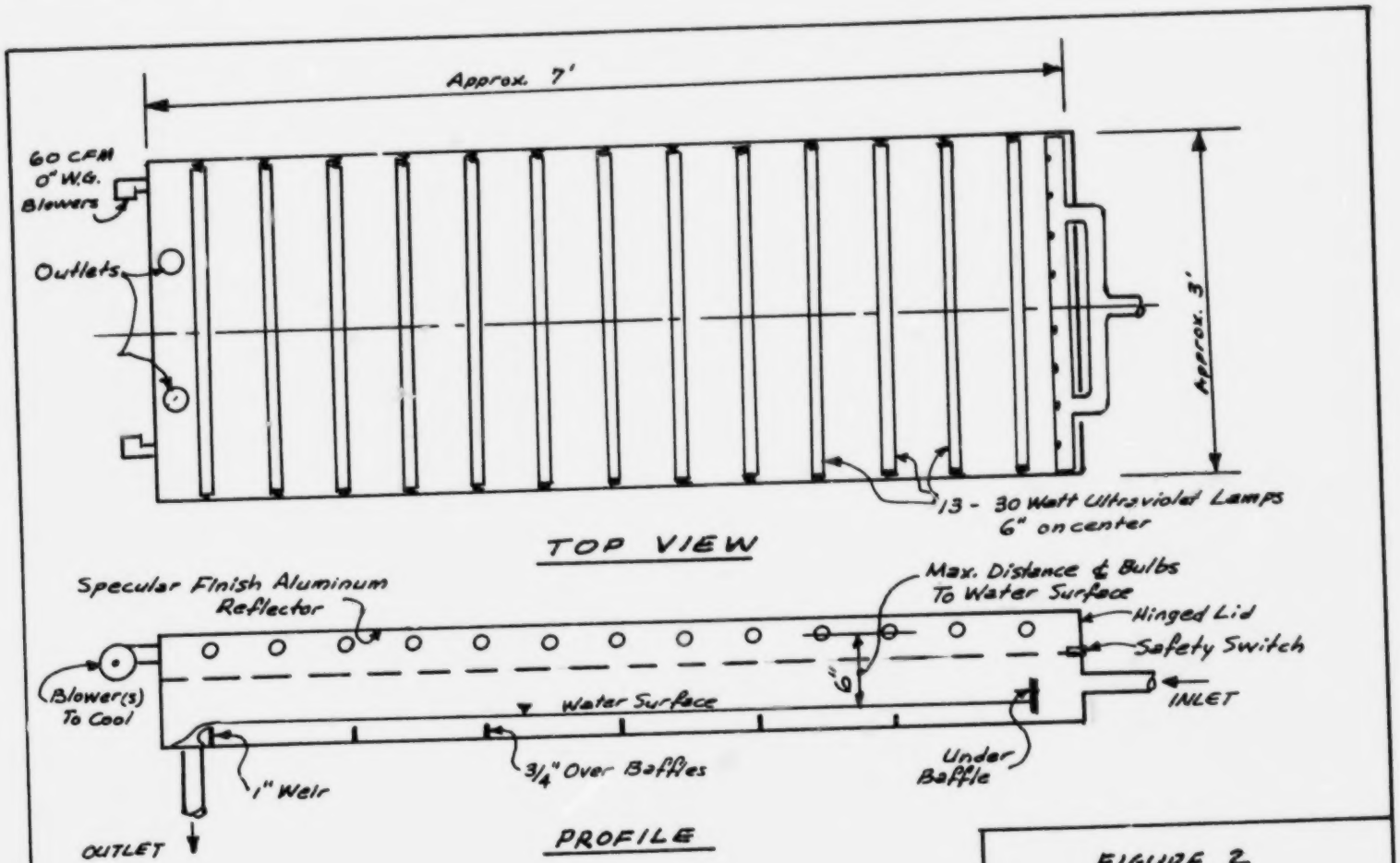
As a general rule depuration plants should not be located where the source of process sea water is affected by (a) industrial wastes or by (b) runoff from farms which use heavy amounts of pesticides.

Bacterial quality of the sea water is used as an index of contamination by fecal wastes. Specifically, coliform and/or fecal coliform organisms are used as indicators of pollution. The raw water source for depuration plants preferably should contain less than 70 coliform MPN/100 ml; but equal to or less than 700 is the maximum median acceptable. In any event, bactericidal treatment of sea water is necessary because of chance pollution. The maximum allowable coliform content of waters contacting the shellfish shall be 1 per 100 ml.

The inactivation or killing of micro-organisms in sea water has been accomplished by several means. Among them are chlorination, ultraviolet light treatment, ozonation and heating. Chlorination is not recommended for treatment since experiments by Galtsoff (1946) and Kelly, et al, (1960) have shown that oysters' feeding activity was reduced even in concentrations of 0.01 mg/liter chlorine as well as in dechlorinated sea water. Heating of sea water is not practical nor recommended since the high temperature (at least 160°F) required means that a cooling process must follow. Furthermore, this heating may affect beneficial biological life (food) as well as add considerably to the cost of the plant. Ultraviolet light treatment has been used successfully in pilot and commercial plants in Europe and the United States. UV treatment is discussed more fully in the next section. Ozone has been used to treat sea water (Violle, 1929) but not extensively. Wood (1961b) reports that ozone requires careful control since ozone, above certain concentrations, may weaken or kill oysters. For fresh water ozone may be bubbled through at concentrations of 1 - 4 milligrams per liter for about ten minutes to produce some disinfection (Fair and Geyer, 1959). Ozone generators, however, require auxiliary equipment such as blowers, piping and filters which must be maintained. Ozonation is fully discussed by O'Donovan (1965).

Special Treatments

Ultraviolet Light: An effective UV water treatment unit has been developed by the Public Health Service at Purdy, Washington (Kelly, 1961b). It is depicted in Figure 2. It may be constructed of plywood coated with an epoxy or polyester compound. The flow limits for this particular unit are shown in Figure 3. A flow of 60 gallons per minute (gpm) should not be exceeded because of hydraulic considerations. Also for this treatment unit, if the absorption coefficient of the sea water is not measured, a flow of 28 gpm should not be exceeded. Multiple UV units located in parallel will offer flexibility for higher flows.



NOTES:
1. Since U.V. Bulbs absorb U.V. radiation minimum recommended spacing is 4"
2. Parabolic reflectors over each bulb will increase efficiency.
3. Unit must be level.
4. Ground the electrical system.

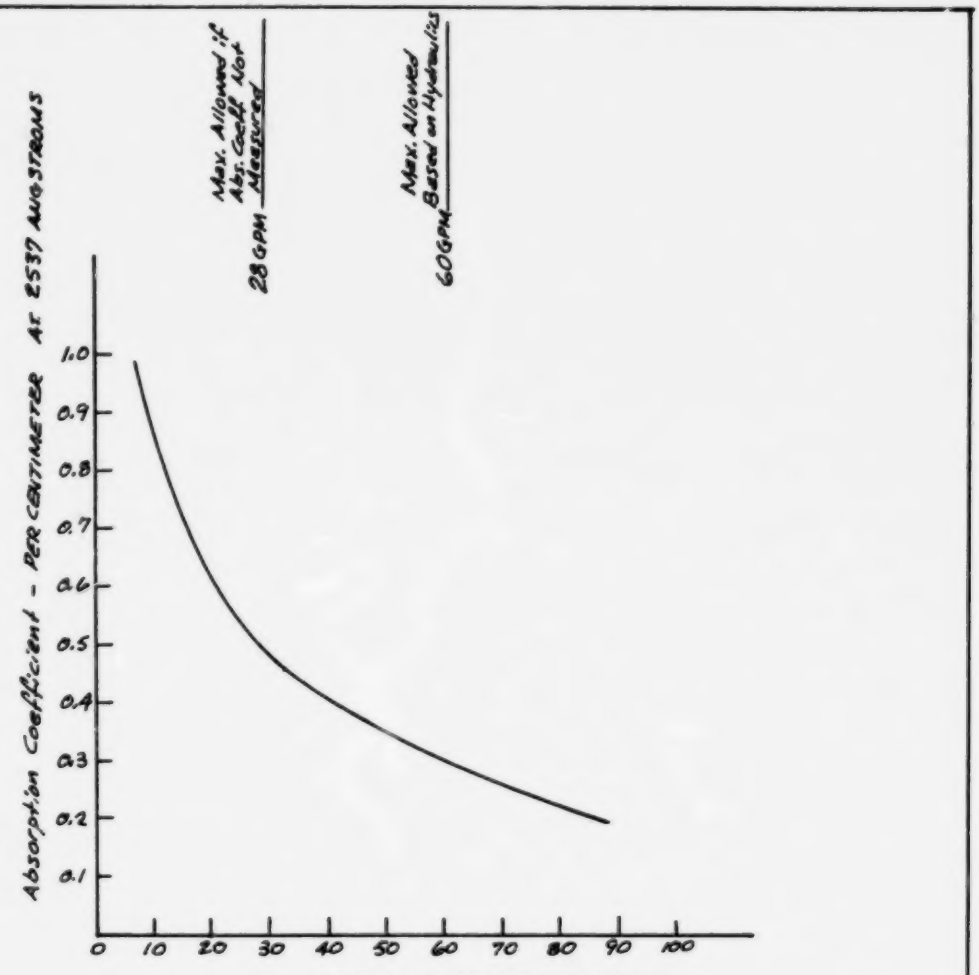
Rev. Jan., 1966

FIGURE 2

ULTRAVIOLET TREATMENT UNIT FOR SEA WATER

PURDY, WASH. DESIGN

OCT., 1964 NERC, FOA, SDF



Not to be exceeded for use in PURDY U.V. Treatment Unit
For a given Absorption Coeff.
and 99.995% Coliform Kill

$$\frac{I}{I_0} = e^{-ad}$$

a = Absorption Coeff. - per cm.
d = Depth - cm
I = Intensity of U.V 2537 A° at depth d
I_0 = Incident Intensity
e = 2.718...

FIGURE 3

FLOW LIMITATIONS OF PURDY U.V. TREATMENT UNIT

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There are commercially built units available with varying flow requirements. Certain manufacturers may assist in custom building UV units for specific applications.

There are four operational items pertinent to the use of this or any other UV treatment unit. They are: 1) bulb monitoring and replacement; 2) maintenance of the unit; 3) safety of personnel; and 4) reliability.

UV tubes should be checked for intensity (commercial meters available) on a monthly basis and should be replaced when they reach a point of 60% efficiency or 7500 hours old, whichever occurs first. A log of intensity must be kept and an orderly numbering procedure for units and bulbs should be established. Figure 4 is an example of a typical log sheet which may be adapted to a particular situation for monitoring of bulbs.

Tubes and reflectors should be cleaned DAILY. Cleaning can be done with a clean damp cloth and dried with a dry cloth.

Signs stating, "Ultraviolet Light - Danger To Eyes - Do Not Look At Bulbs Without Eye Protection," must be displayed in full view to personnel and casual visitors. Conjunctivitis is caused by looking at lit UV bulbs, and although it is not usually permanent, it is extremely painful (Phipps and Hanel, 1960). Skin protection, especially to the face and hands should be procured for personnel monitoring the bulbs. Eye protection consists of ordinary glasses with solid side pieces. Face protection is afforded by many clear plastics but not all. Plexiglass (lucite) transmits about 5% of 2537 Angstrom light. Cellulose acetate butyrate (0.41 mm) will transmit 2537 A° to some degree. Skin protection for the head is afforded by a hat, and on the hands by thick gloves.

An automatic shut-off switch is a required safety feature to the Purdy Unit (or any unit) to break the electric circuit when the lid of the unit is raised.

The continuity of operation of any UV unit during a depuration run must be monitored. Since power failures occurring in the absence of personnel may not be noticed, the shellfish may not receive acceptable water during some of the process time. Inexpensive electric current on-off recorders are available to serve not

Figure 4. TYPICAL LOG SHEET FOR UV TUBE MONITORING

UV TREATMENT UNIT NO. _____

Date Checked	Checker	Ultraviolet Intensity--micro watts/cm^2 @ 1 meter BULB NO.							Remarks (Record Tube Life in Hrs.)
		1	2	3	4	5	. . . 14	15	

only as a measure of continuing operation but also as a measure of bulb life. Electrical clocks installed in line with the different UV units, synchronized with the main power supply offer another means of determining how long UV units were not in operation. UV photo cells and intensity meters that may activate warning devices or electric controls are useful. However, these must be used with caution since the Purdy Unit has 13 tubes separated over a distance of about six feet and monitoring all or some of these by means of photo cells may not be practical.

Filtration: It was stated earlier that filtration as a means of removing turbidity was not highly recommended since microbiological life used as food by shellfish also may be filtered out.

Bacterial reduction can be accomplished by sand filtration. At Biddeford Pool, Maine, (Goggins, et al, 1964), experiments with well points driven 4 - 6 feet deep in sand below the low water mark, showed that coliform values in the overlying sea water averaging about 760 MPN/100 ml were reduced by 92%. Sole reliance on filtration for bacterial removal is not recommended, but in conjunction with UV treatment is acceptable provided it is shown that filtration does not affect shellfish activity and feeding.

Time for Depuration and Expected Bacterial Results

The time required for depuration is a function of 1) the initial pollutional load in the shellfish; 2) the final bacterial levels desired; and 3) the environmental conditions in the depuration system.

Item 3) will be kept relatively constant. Depuration plants will be operated to produce the environmental conditions described previously for optimum shellfish activity. The interdependence of the remaining factors; time, initial levels, final levels can be seen from the discussion in Appendix II.

Based on the discussion in Appendix II, the expected bacterial levels of the various species after 24 hours and 48 hours under different conditions are given in Table 3. The main presumption is that for public health assurance, depurated shellfish harvested from restricted areas should attain the same low bacterial levels of shellfish harvested from approved or conditionally approved areas.

The expected bacterial levels of approved area shellfish depurated for 24 hours are given in the first column. Generally speaking, to attain these same levels, shellfish harvested from waters containing about 200 coliform MPN/100 ml must be depurated for 48 hours. For the restricted areas where the coliform content exceeds 200/100 ml but is less than 700/100 ml, longer times than the 48 hours may be necessary to attain the same low levels.

It is recommended that, if experimentation is not done for a given locale to show the time required to attain certain levels (e.g., State Standards), the shellfish harvested from waters in the range of 70 - 200 coliform MPN/100 ml be depurated for 48 hours, and that shellfish harvested from waters in the range of 200 - 700 coliform/ 100 ml be depurated for 72 hours. Shellfish harvested from waters containing more than 700/100 ml coliform must not be subjected to the depuration process described in this report.

Controlled Storage

Shellfish which are received at a depuration plant and cannot be processed immediately should be placed in controlled storage. The most important aspect of a controlled storage for untreated shellfish is that the shellfish must not be shocked such that the depuration process is inhibited. Therefore it is recommended that the controlled storage for untreated shellfish include a controlled temperature at about the same levels of the process sea water if the shellfish are to be processed within 24 hours of harvesting*. If shellfish are to be stored for longer

---

*To avoid bacterial multiplication or spoilage of the shellfish, the maximum storage temperature should be 70°F.

Table 3. TIME FOR DEPURATION AND EXPECTED BACTERIAL QUALITY AFTER DEPURATION

	SOURCE OF SHELLFISH					
	Approved or Conditionally Approved Area			Restricted Area*		
Shellfish	Time of Depuration Hours	Expected Coliform MPN/100ml	Expected E.C.+ MPN/100ml	Time of Depuration Hours	Expected Coliform MPN/100ml	Expected E.C.+ MPN/100ml
Soft Clam	24	200	<20	48	200	<20
Hard Clam	24	<20	<20	48	<20	<20
Oyster	24	23	<20	48	23	<20

*The coliform content of the water in the restricted area to achieve the expected coliform results after 48 hours of depuration will be about 200 coliform MPN/100 ml.

than 24 hours before zero hour then the controlled storage requirements should conform to standards set for the National Shellfish Sanitation Cooperative Program. A gradual change of temperature from the storage temperature to the depuration temperature may then be necessary.

Shellfish which have been thru the depuration process should be placed in cold storage if they are not to be distributed or consumed immediately. The temperature requirements for this storage must meet the requirements of the National Shellfish Sanitation Program.

Laboratory Control and Sampling Procedures

Every controlled process must have quality tests to determine if standards are being met and if controls are effective. Since depuration of shellfish is a controlled process, minimum sampling procedures are described as follows:

I. Sea Water

A. Raw Sea Water at the Plant

1. Frequency - once per day for routine tests.
2. Routine Tests: Temperature, Turbidity, Salinity.
3. Other Tests: Coliform and E.C. MPN, once/week.

B. UV Treatment Effluent

1. Frequency - a) if one UV unit - twice per day.
   b) if more than one unit - once/unit/day.
2. Type of Test: Coliform.

C. Sea Water in or from Tanks*

1. Sample the 24-hour tanks' water.
2. Number of tanks to sample:

Total Tanks in Plant	Arbitrary Number to Sample
4	4
10	5
>15	6

3. Type of Tests: a) Dissolved oxygen
   b) Coliform

D. Harvest Area Waters**

1. Frequency of bacterial sampling: At least 15 samples through course of a harvest season.
2. Type of Tests:
   a) Temperature, Salinity, pH.
   b) Coliform and E.C.

II. Shellfish: From each harvest area should be sampled from randomized harvesters lots as follows:

A. Time for Sampling

1. As delivered to plant.
2. At zero hour if storage time exceeds 24 hours.
3. At 24 hours of depuration.
4. At 48 hours of depuration.
5. At 72 hours (if necessitated by choice of allowing 3 day depuration).
6. In storage, if storage time after depuration exceeds 24 hours (recommended).

B. Type of Test

1. Coliform MPN/100ml.
2. E.C. gas positive MPN/100ml.

*Desirable location near effluent of tank.
**This is conducted under the Sanitary Survey part of a shellfish program.

3. Recommendation: Since shellfish replace their mantle fluid with the clean depuration water within a short time after being put in the water, it is recommended that MPN tests be conducted on the drained meats. This is a slight modification to the APHA standard method. The results of Table 3 are based on the APHA method which will give lower coliform results because of dilution by the mantle fluid.

DESIGN CRITERIA

PRACTICES

Sea Water Flow Rates

Sea water flow is an important consideration in the cost of a depuration plant. On it are dependent, the size and number of ultraviolet light treatment units, pump size and capacity, pipe size and arrangements, and heat exchange capacity. The prime consideration of the minimum allowable sea water flow is the dissolved oxygen requirements of the several species of shellfish.

It is recommended that the minimum flow rate for any species in depuration plants be 1 gallon per minute per bushel being depurated. This is based on an analysis of dissolved oxygen requirements of certain species and recommendations by others. If, for example, the total plant capacity is 200 bushels, then a sea water supply system for 200 gpm must be designed.

Flow rates must be monitored and recorded for each tank. The methodology for accomplishing this is largely dependent on the size of the tank. Overflow devices such as weirs, strategically placed in troughs, along with staff gages, are useful for a wide range of flows. Parshall flumes designed into the open channels serve the same purpose. Venturis, orifice plates or certain types of flow meters can be inserted in pipe lines to measure flow by pressure drop principles. However, these offer constrictions in pipe lines and frequent cleaning may be necessary to maintain calibration of the device.

Tank Washing

After each 24 hours that the shellfish are in tanks, the sea water in the tanks should be drained out and the shellfish should be hosed down thoroughly with either potable tap or treated sea water at about the same temperature as that of the depuration. Feces and pseudofeces and any other waste matter should be flushed out from the tank.

The 24-hour washdown of shellfish and tanks is extremely critical to successful depuration. Experimental evidence shows that shellfish feces are the focal point of any pathogenic micro-organisms that were in the gut of the animal. Without an adequate hosing and flushing, the sea water in the tanks will become contaminated and the shellfish may repollute themselves with their own wastes and depuration is not attained. This procedure should not be carried out more frequently than once a day since shellfish activity is interrupted each time. This procedure must be carried out in closed or open systems.

A highly recommended alternate procedure consists of a short-time washdown of the shellfish and tank surfaces with a chlorine residual of about 10 mg/l, followed by a hosing down with an unchlorinated acceptable water to remove all traces of the chlorine.

After hosing, the tanks should be filled with treated sea water as soon as possible; in any event within an hour.

Shellfish Washing and Culling

Washing and culling are two operations which are required for satisfactory depuration. Shellfish must be washed and culled before and after depuration. When shellfish enter plants, they are frequently covered with mud and bottom sediment which may contain pathogenic organisms. This material must be removed from the shells so that the sea water system will not become contaminated. Broken-shelled and dead shellfish must be removed from the lots which are to be depurated. Dead shellfish decay in water and the accompanying bacterial multiplication will contaminate the sea water system. Broken-shelled shellfish cannot be relied upon to depurate.

Washing must precede culling because (1) the washing process may break shells; and (2) culling is better carried out if clean shellfish are inspected.

After depuration, the feces and pseudofeces which contain micro-organisms must be washed from the shells. A few dead shellfish which were missed inadvertently may reveal themselves after depuration and therefore culling is required again.

The washing operation for the treated and untreated shellfish must be carried out with separate facilities. The reasons include the prevention of mixing, inadvertently, the treated and untreated lots, and also to prevent the contamination of treated shellfish with any sort of contact with the untreated shellfish. In large plants, **mechanical washing (of shellfish) is highly desirable.** In smaller plants (50 bushels or less) manual washing may be adequate provided there are separate facilities for untreated and treated lots.

It is recommended that the washing and culling process be carried out as mechanically as possible so as to produce uniformity of results. The following criteria can be used for designing a mechanical device:

1. A device to feed shellfish into the machine to minimize breakage.

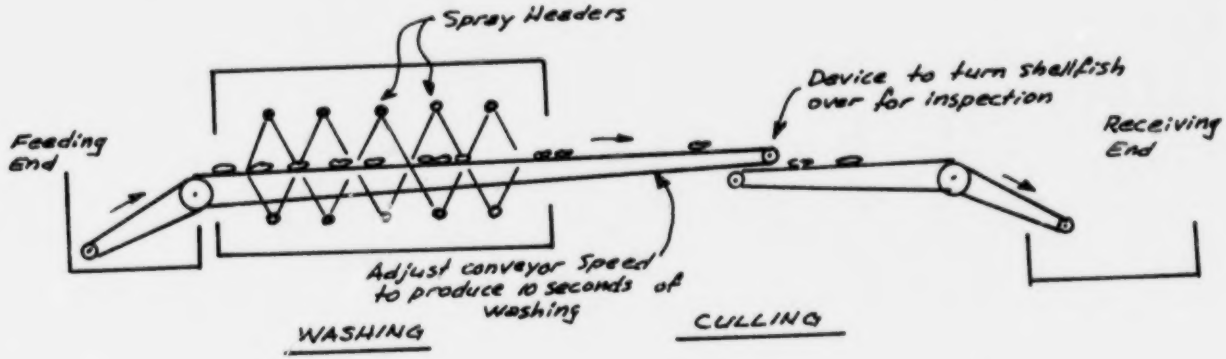
2. Jet spray washing covering both valves of all shellfish.

3. Ten seconds of washing of a shellfish under pressure spray of at least 20 psi with fresh or treated sea water of drinking water quality for coliform (1 MPN/100 ml). A better but not required operation includes a chlorinated wash (10 mg/l Cl_2) followed by an unchlorinated water rinse.

4. Capability for observation of both valves of every shellfish by a culler and easy removal of broken, etc., shellfish by culler.

5. Easy placing of shellfish into a container after washing and culling. (Under certain circumstances shellfish may be placed into baskets prior to washing, **e.g., after culling.**)

Figure 5 depicts, schematically, a device used in pilot and commercial plants in Maine and Massachusetts. (Designed and developed by the State of Maine, Sea and Shore Fisheries; refer to Goggins et al, 1964.)



NOTES: 1. Wash water should meet PHS Drinking Water Standards. If machine uses recirculated water, treatment is required (Chlorine is recommended)

2. Sea water or Tap Water may be used.

FIGURE 5
SCHEMATIC OF
A SHELLFISH WASHING AND
CULLING MACHINE
(After State of Maine)

OCT., 1964 NERC, FOA, SQ7

Waste Handling

A list of some wastes produced in a depuration plant and the means to cope with them are as follows:

Type of Wastes	Disposal Process
Human (feces, urine)	Sanitary sewers; private disposal systems.
Dead Shellfish	Discard to labelled containers containing a denaturant (cresol, e.g.) or bleach to prevent inadvertent human consumption. Ultimate disposal as in "other wastes" below.
Tank Washings	Shellfish feces, scum, sludge should be drained to sanitary sewers (shellfish feces are contaminated).
Other Wastes	Garbage, trash, etc. - local requirements.

CONSTRUCTION

General

The general construction of depuration plants must conform to most of the requirements for shucking plants enumerated in Part II of the Manual of Operations of the National Shellfish Sanitation Program, "Sanitation of the Harvesting and Processing of Shellfish," 1965 Revision.

The requirements center on the protection of shellstock from contamination. Plants should be of sufficient elevation to prevent flooding of tanks by high tides. Floors shall be constructed of concrete, coated if necessary to prevent leaching, and graded to suitably located drains and drain troughs. Floors and walls shall be smooth for easy cleaning. Joints should be impervious. Corners should be coved. Insect and rodent ingress shall be prevented by suitable screening and construction. There shall be no cross-connections among the several piping systems (both pressure and drainage) in a depuration plant.

Back siphonage shall be prevented by airgaps **or other devices.**

Other items concerning employee hygiene are covered in Part II of the Manual.

Lighting

Lighting requirements vary with the different areas. Of importance, however, is the effect on shellfish. Most shellfish have a light-sensitive mantle edge. To insure constant conditions for the shellfish it is necessary to maintain at least 10 foot-candles of illumination at the water surface level of the depuration tanks throughout the day. These water surfaces should not be subjected to the variations of direct sunlight. Washing & culling areas require 50 foot-candles. Lighting requirements for other types of areas (because of frequency of employee usage) are described in Part II of the Manual or in suitable architectural handbooks.

Materials

All materials contacting the process sea water shall be classified in the usual sense as inert and non-corrodable, as well as non-toxic to shellfish.

Pipes and Piping Systems

There are many types of material which are acceptable for piping for depuration plants. Some have certain advantages over others. Many are within the same price range of each other. For some material the pipe is relatively inexpensive, but the cost of valves is high; and vice versa. The following table is to be used solely for informational purposes and not strict adherence. Regional costs and requirements may differ significantly.

Intake and exterior lines should be pitched for draining, flushing and cleaning. Drainage of pipes is most important for those areas where freezing occurs. Pipes near electrical equipment should be insulated to prevent formation of condensation. Ends of intake lines must be accessible for cleaning. Intake lines fastened to piers are desirable. Iron intake lines are unacceptable as they may contribute rust to a system. Vitrified clay pipe is relatively inexpensive and with sealed joints is acceptable for sea water drain lines.

Table 4. PIPE ACCEPTABLE FOR SEA WATER IN DEPURATION PLANTS

Type & Description	2" Size** Relative Cost per Linear ft	Relative Cost of 90° Ell	Remarks
Polyethylene, Schedule. 40*	1.0	1.9	Low pressure.
Cast Iron, Heavy	1.5-2.0	1.0	Not acceptable for recirculating systems.
Polyvinyl Chloride, Schedule. 80	1.5-2.0	1.8	Cost of valves, high.
Asbestos-Cement	2.0		Not acceptable indoors--pervious. All right for exterior intake and drain lines.
Saran	2.0-2.5	1.4	Low pressure, valves relatively inexpensive.
Type 304 Stainless Steel***	2.5-3.0		Not acceptable for recirculating systems.
Epoxy	2.5-3.0	7.0	
Glass Reinforced Polyester	3.1	11.0	100 psi pipe
Type 316 Stainless Steel***	3.0-3.5		Not acceptable for recirculating systems.
Saran-Lined Steel	3.5-4.0	12.2	
Glass, Regular	4.0	5.9	
Rubber-Lined Steel	6.0	17	
Penton-Lined Steel	10		

*This pipe in 1963 cost about $0.71/linear foot.
**To obtain cost estimate, multiply current price of polyethylene by factor given in this column.
***Note: Stainless steels are not in general always useable from the corrosion standpoint tor sea water. **Consult fabricators for suitable formulations.**

Depuration Tank Construction and Tank Linings

The gross volume of tank for a given capacity of shellfish is important to depuration for several reasons: they are stacking and arrangement of baskets and the shellfish feces and pseudofeces removal problem. The following Table 5 presents the recommended factors derived in Appendix III:

Table 5. TANK SIZING FACTORS

Shellfish	Gross Tank Size Factor
Soft Clam	5 cubic feet of Tank/Standard U.S. Bushel
Hard Clam and Oyster	8 cubic feet of Tank/Standard U.S. Bushel

Tanks should have sturdy foundations so that vibrations, caused by vehicular traffic outside the plant, or plant operations themselves will not affect shellfish activity. Tanks may or may not require insulation depending on construction material, type of sea water system and whether conservation of the heat or cold is economical. Outdoor tanks are used in Europe. Outdoor tanks are acceptable but must not be located where they are subject to pollution.

The physical shape of the depuration tanks should be such to prevent short-circuiting of the sea water. If rectangular, length to width ratios should be from 2:1 to 4:1. Depth of tanks will vary with the number of baskets (see next section) spaced on top of each other. The most efficient depth of water would be about 24 inches to accommodate two baskets.

The tanks should be so designed that scum and sludge (shellfish feces and pseudofeces, sand, grit, vegetation, etc.) can be easily removed or flushed out. The bottom should be sloped longitudinally 1/4 to 1/2 inch per foot from inlet to outlet end. Lateral sloping is recommended if tank width exceeds 10 feet. Floating matter and scum may be removed by a board which is periodically moved across the surface of the water to a collection trough.

Circular tanks are acceptable provided the features described above are incorporated in the construction.

Tanks may be made of concrete, wood, fiberglass or any substance which is inert or which can be coated if necessary. Coatings which have been used in sea water systems and aquaria include: epoxy resins, powdered polyesters, vinyl bituminous water-tank paint and paraffin for concrete. Cost for epoxy coatings are in

the order of $0.10 per sq.ft. These coatings are not only for waterproofing but for providing a smooth hard, non-porous surface for cleanability. Linings include fiberglass and polyethylene. Prefabricated fiberglass tanks are available commercially and if procured in sufficient numbers, may be less expensive per unit, than custom constructed and coated tanks.

Pumps

Use of centrifugal pumps is recommended but not required. They are more generally used than other types (reciprocating, screw, gear, etc.). These pumps should have rising characteristic curves (head vs. flow rate) and non-overloading impeller. Intake pumps should be located for low suction lift so as to minimize priming problems as well as cavitation. Pumps should be provided in duplicate for continuing service, and arranged in parallel (not in series). Pumps with vertical motors set on a pier and pump heads submersed in the water offer a desirable intake pumping arrangement but usually require a pier or intake structure. There are commercially available self-priming centrifugal pumps of most sizes and pump-motor arrangements.

Pump volutes and impellers shall be of non-toxic (to shellfish or people) material. Pump components of compressed graphite, hand-rubber and certain types of plastic linings are commercially available.

Baskets

All shellfish should receive the same quantity and quality of sea water. Therefore, the shape, size and arrangement of holding baskets are important factors. **(See Table 5.)**

The shellfish holding baskets should not be over six inches deep for oysters and hard clams. For soft clams, baskets of 8-inches depth are acceptable based on experience at the Newburyport, Massachusetts, depuration plant.

A mass of shellfish obstructs the free flow of water. The shape and arrangement of baskets should allow water to flow all around the baskets. Baskets must be meshed to allow a

flow-thru feature. The minimum distance between levels and horizontal spacing of baskets shall be 3 inches.

On the basis of current design, not more than four baskets should be stacked on one another because of the shellfish washing problem. Feces removal by overhead spraying will be difficult thru greater layers. Spray headers located at strategic depths to insure coverage of all baskets of shellfish will allow greater stacking of baskets in tanks.

PLANT SIZE AND ESTIMATED DESIGNING FACTORS

Table 6 presents factors on building, equipment, and personnel requirements. The values presented are approximate but can be used effectively for initial decision making on plant size. The plant capacity means the total number of bushels that a plant can hold in depuration tanks.

Floor areas and tank capacities were derived from an analysis of hypothetical plant layouts. Flow of sea water was derived using the 1 GPM/bushel factor. The number of Purdy UV units was derived from an assumed allowable flow of 35 GPM per unit. Controlled storage volume assumes a one-day storage plus 25%. The total personnel includes at least one supervisor and one laboratory technician. (Personnel requirements will be subject to plant design and automation.) Sea water heating requirements are based on heating sea water from 32°F to 54°F (0° - 12°C) in an open system. For the larger plants a certain portion of the sea water should be recirculated to minimize heating costs.

Table 6. DESIGNING FACTORS FOR DEPURATION PLANTS

Plant Capacity Bushels	Floor Area of Building Without Lab. Sq.Ft.	Floor Area of Building With Lab. Sq.Ft.	Total Tank (1) Capacity Cu. Ft.	Flow of Sea Water GPM	No. of Purdy UV Units	Controlled Storage Volume Cu.Ft.	Wash (2) & Cull Machines	Total Personnel (Minimum)	Sea Water(3) Heating Requirements Lbs. Steam Per Hour	Approximate Daily Output Bushels
50	500	700	400	50	2	80	0	3	570	20-25
100	800	1000	800	100	3	155	2	3	1100	35-50
200	1500	1700(4)	1600	200	6	310	2	3	2300	70-100
400	3000	3200	3200	400	12	630	2	4	4600	130-200
800	5700	5900	6400	800	23	1250	2	4	9100	270-400
1200	8500	8700	9600	1200	35	1900	2	5	13600	400-600

(1) For Quahogs and Oysters: Use 62.5% of these values for Soft Clams.

(2) The type designed by Maine Department of Conservation. For small plants see text.

(3) Open system. (Multiply these values by 940 ((for 35 psia saturated steam)) to obtain BTU/hour.)

(4) Larger plants may require more than 200 square feet of laboratory space.

APPENDICES

TABLE OF CONTENTS

Appendix No.		Page No.
I.	SEA WATER QUALITY AND QUANTITY	39
	A. General	39
	B. Temperature	39
	C. Turbidity	40
	D. Hydrogen Ion Concentration (pH)	48
	E. Salinity	49
	F. Oxygen and Flow Rate	52
	G. Metals	56
	H. Pesticides	56
	I. Marine Toxins	58
	J. Further Notes on Feeding and Activity of Mollusks	58
II.	DERIVATION OF BACTERIAL LEVELS CORRELATED WITH TIME FOR DEPURATION	60
	A. Experimental Depuration Runs	60
	B. Depuration of Salmonella	70
	C. Growing Area Surveys and Laboratory Results	70
	D. Derivation of Expected Levels of Attainment	81
III.	ESTABLISHMENT OF SOME PLANT DESIGN FACTORS	86
	A. Sea Water Flow	86
	B. Tank Loading Information	86
IV.	SHELLFISH DEPURATION AND VIRUSES	87
V.	SYNOPTIC HISTORY OF DEPURATION AND DEPURATION RESEARCH	92
VI.	ECONOMICS, FINANCING AND MANAGEMENT OF DEPURATION PLANTS	96
VII.	SHELLFISH QUALITY STANDARDS IN DEPURATION PLANTS	103

LIST OF FIGURES

Figure No.	Description	Page No.
A1	Activity of the Hard Clam.....	41
A2	Pumping Rate of Oysters vs. Temperature.....	42
A3	Relative Coliform Uptake for Shellfish.....	44
A4	Effect of Turbidity on Pumping Rate of Oyster.....	47
A5	Effect of Salinity on Pumping Rate of Soft Clams.....	51
A6	Oxygen Uptake of Oysters.....	54
A7-A14	Depuration Runs on Shellfish.....	61-68
A15	Depuration of Soft Clam Salmonella Reduction.....	71
A16-A19	Coliform-Shellfish-Water Relations, Growing Areas.....	72-75
A20-A23	Coliform-Shellfish-Water Relations, Laboratory.....	76-79
A24	Depuration of Polio III Virus.....	88
A25	Fate of Coxsackie B5 Virus in Hard Clams....	90
A26-A29	Cost Curves vs. Plant Sizes.....	97-98
A30	Hypothetical Example of True Bacterial Depuration.....	106
A31	Hypothetical Trend of Quality Control in Depuration Plants.....	108
A32	Typical Depuration Plant-Harvest Area Relations.....	110

LIST OF TABLES

Table No.	Description	Page No.
A1	Occurrence of Spawning.....	45
A2	Allowable Temperature Ranges.....	46
A3	Algae and Gill Clogging of Oysters.....	46
A4	Effect of pH on Pumping Rate of Oyster.....	49
A5	Effect of pH on Oxygen Uptake.....	49
A6	Acclimatization to Low Salinities.....	50
A7	Minimum Survival Salinities for Shellfish.....	52
A8	Oxygen Uptake of Oysters.....	53
A9	Oxygen Uptake of Oysters.....	53
A10	Uptake of Pesticides by Shellfish.....	57
A11	Retention of Pesticides in Shellfish.....	57
A12	Results of Depuration Runs.....	69
A13	General Levels of Coliform Remaining.....	60
A14	Coliform Organisms - Shellfish Water Relations.....	80
A15	Attainment Levels of Coliforms for Depurated Shellfish From Approved Areas.....	82
A16	Calculations on Levels of Attainment - Restricted Areas.....	83
A17	Expected General Levels for Restricted Area Shellfish.....	84
A18	Coliform Content in Harvest Area Waters - Restricted Area.....	84
A19	Tank Size and Loading Factors.....	86
A20-A24	Cost-Plant Size Relationships.....	99-101

APPENDICES

I. Sea Water Quality and Quantity

A. General

The purpose of the following discussions is to show how the guidelines, presented earlier, have been derived from the basic biological principles found from research. Only a small portion of the works cited were directed toward depuration. However, the results of the research can be applied as criteria for depuration, since the process involves high feeding rate and activity of the animals.

B. Temperature

1. Soft Clam. Marston (1930) experimenting on feeding rate of soft clams at various temperatures showed that Bacillus prodigiosus was ingested at 37°F and 80°F at about the same rate. She also concluded (referring to the Newburyport, Massachusetts, Purification Plant) that artificial heating of sea water for soft clams was not necessary since clams cleansed themselves as readily at 37°F as at 80°F.

In the Massachusetts (1947) Report it was stated that temperatures below 50°F were believed to be detrimental to purification. However, it was also concluded that within certain limits the temperature at which the clams were accustomed was the optimum temperature for their depuration.

Arcisz and Kelly (1955) showed in depuration studies that soft clams cleansed themselves of coliform bacteria and Salmonella schottmeulleri at temperature ranges of 2.5 - 4°C (36-39°F), 3.5 - 4.5°C (38-39.6°F), 13°C (55°F), and 15°C (59°F) equally as well.

2. Hard Clam. Pierce (1950) stated that at below 5°C (41°F) hibernation of the hard clam occurs and that at 22°C (72°F) the hard clam actively pumps for 96% of the day.

Feng (1963), using several activity factors as a means of describing relative activity as a function of temperature, has shown that the greater activity occurs in the range of 8°C (46°F) to 20°C (68°F). Figure A1 depicts the information.

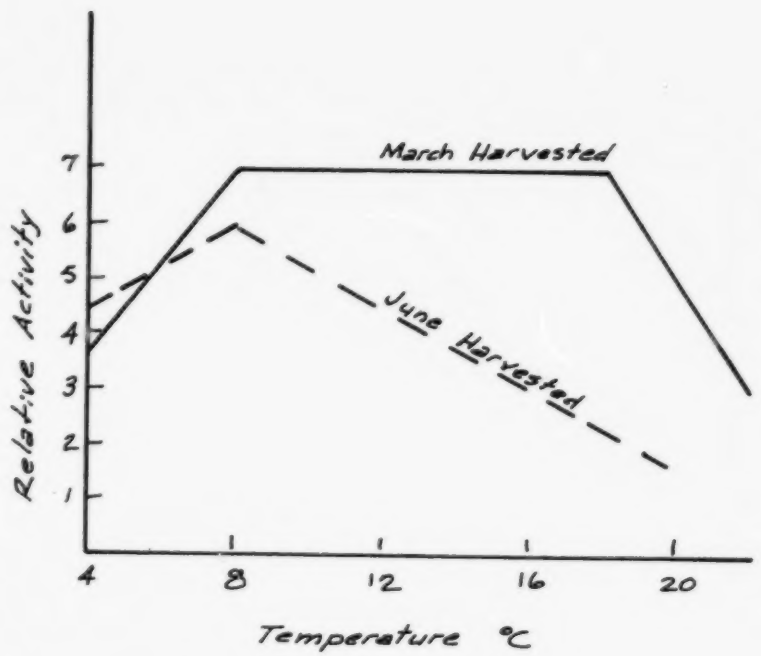
Feng (1965) presented a chart giving temperature-salinity contours for various degrees of activity for the hard clam. There seems to be no one point or zone of a temperature-salinity combination at which activity is at an optimum.

3. Oyster. Loosanoff (1958) has shown a steady increase of pumping rate of water by eastern oysters (C. virginica) up to about 30°C (86°F). Figure A2 presents the data through which a curve has been fitted by eye. From this curve it appears that a minimum temperature for depuration would be about 10°-15°C (50°-59°F) and a maximum temperature would be about 29°C (84°F).

Nelson (1923) described sharp decreases in ciliary activity of New Jersey Oysters (C. virginica) at temperatures of 4°-6°C (39°-43°F) and no feeding at temperatures less than 4°C. He stated that at temperatures above 20°C (68°F) the oysters pass water for 20 hours. Galtsoff (1928) stated that there was no water current in C. virginica at less than 5°C (41°F). He described an optimum temperature range of 25°-30°C (77°-86°F).

According to Elsey (1936), the Pacific Oyster (O. gigas) pumped 1.0 quart/hour at 1°C (34°F) and 5.5 quarts/hour at 25°C (77°F). Hopkins (1931) described Olympia Oysters (O. lurida)as hibernating with respect to gill activity at temperatures of 4°-6°C (39°-43°F). Hopkins (1933) concluded that the gills of O. gigas appeared most efficient at 25°C (77°F), but that the degree of shell openness was greatest at 20°C (68°F).

4. Bacterial Uptake as a Function of Temperature. From the results of a series of experiments by Kelly (1956) showing bacterial accumulation of the soft clam, the hard clam and the eastern oyster indigenous to the northeast, relative accumulation has been shown to be dependent on temperature and bacterial concentration in the water. The results have been

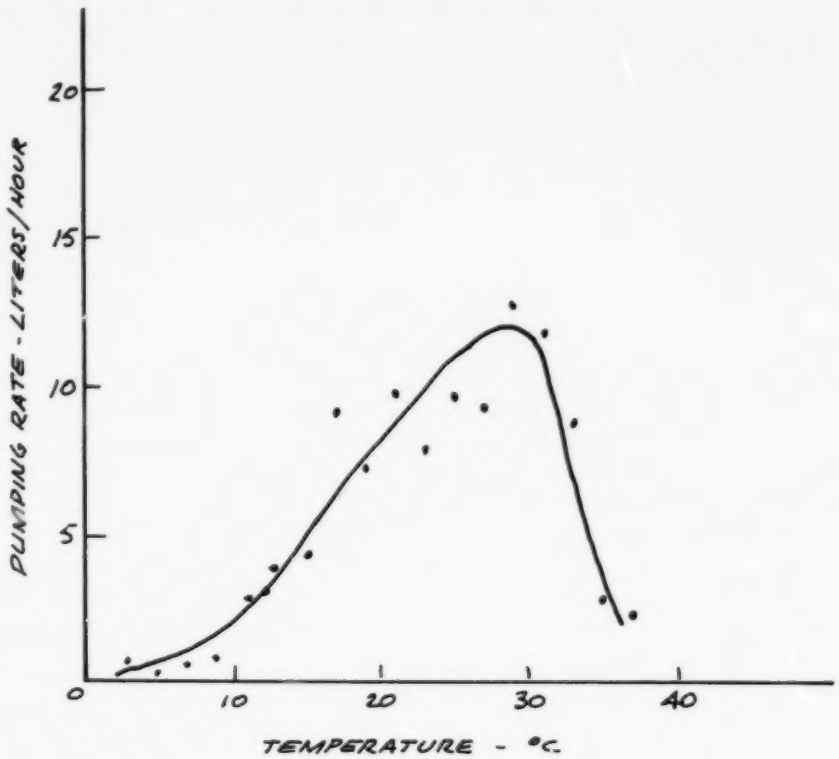


After Feng (1963)

FIGURE A1

ACTIVITY OF THE HARD CLAM MERCENARIA MERCENARIA

OCT., 1964 NERC, FOA, SOFT



After Loosanoff (1958):
Pumping rates for
groups of oysters.

FIGURE A 2.
PUMPING RATE OF OYSTERS
VS. TEMPERATURE
OCT., 1964 NERC, FDA, SAF

reduced to relative uptakes which are presented in Figure A3.

In general, for these particular northeast shellfish, the highest degree of uptake was in the temperature range 8°-17°C (46°-63°F). For the soft clam, the difference in the relative values for the three temperature ranges is not large. However in the cases of hard clams and eastern oysters there is a marked difference in relative uptake for the temperature ranges indicated. Relative uptake dropped for all shellfish in the 20°-23°C (68°-73°F) range.

Kelly (1960) showed similar bacterial uptake trends for Gulf Coast Oysters (C. virginica). Refer to Figure A23.

5. Spawning. The occurrence of spawning during depuration may or may not be a hindrance to the efficiency of depuration. A report of incidental spawning during depuration runs on hard clams by Canario and Tubman (1965) revealed no difference in bacterial reductions.

Galtsoff (1964) describes the various functions of the organs of a female oyster during spawning. The spawning is complex in that the gills, nervous system, mantle and adductor muscle in addition to the ovary are called into play. The more critical consideration is that the mantle edges close on each other and the entrance to the gill cavity is closed off except for a slight opening through which the eggs are released. Feeding must therefore cease during the spawning. Usually spawning time is described as about one hour for a given female oyster.

For a tank full of oysters, many, if stimulated by the proper conditions, will spawn during the entire depuration run. Not every oyster will spawn at exactly the same time. Davis and Chanley (1955) described that even under the ideal laboratory conditions of spawning dishes, occurrence of spawning of oysters and clams was not uniform. Clams spawned in spurts while oysters spawned more uniformly. Ninety percent of a certain batch of oysters spawned within an hour while only 35% of clams spawned in the same time. Nelson (1927) found that for C. virginica held at 20°C, an increase of 2°C can induce spawning within 2 - 3 hours. Nelson (1928) described the temperatures at which spawning can occur for various species. The results are given in Table A1.

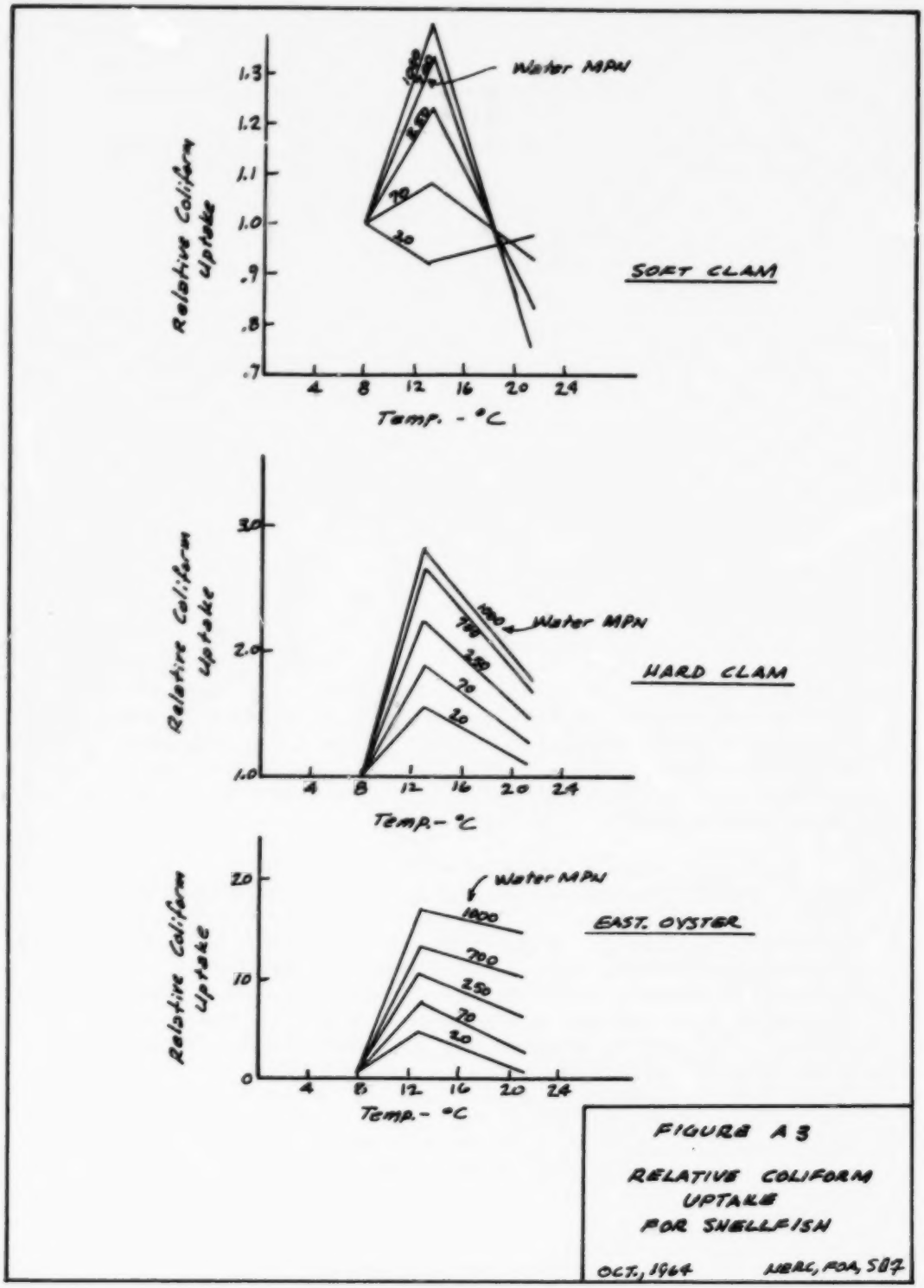


FIGURE A 3

RELATIVE COLIFORM UPTAKE FOR SHELLFISH

OCT., 1964 NERC, FDA, SBT

Table A1. OCCURRENCE OF SPAWNING

Species	Temperature
Soft Clam (*Mya arenaria*)	10°-12°C (50°-54°F)
Hard Clam (*Mercenaria mercenaria*)	25°C (77°F)
Mussel (*Mytilus edulis*)	10°-12°C (50°-54°F)
Oyster (*C. virginica*)	20°C (68°F)
Oyster (*O. lurida*)	16°C (61.5°F)

A qualification for this list is that there are geographical differences in the species-temperature relationships. Also, depending on the geographical area, there may be more than one spawning period for a given species and that the exact time of year for spawning will vary from place to place.

According to Belding (1930) the spawning season of soft clams occurs later in the year the further north the location along the Atlantic Coast. In New Jersey spawning may occur in May while in northern New England, in July or August. Shaw (1963) describes the two spawning cycles of soft clams that occur in Chesapeake Bay. Galtsoff (1964) mentions how the Gulf Coast oyster (*C. virginica*) may spawn over a period of several months of the year while in the north, the season lasts for only 4 - 6 weeks.

Spawning occurrences are drawbacks to depuration in closed systems. The water becomes turbid and therefore UV light penetration is reduced and therefore the chance of a contaminated system is increased.

6. Conclusions. The above information shows that allowable maximum and minimum temperature values for the process sea water is dependent on species and other circumstances. To insure high activity and feeding, therefore, the following general temperature ranges are recommended:

Table A2. ALLOWABLE TEMPERATURE RANGES

	Minimum	Maximum
Soft Clam	35°F (2°C)	68°F (20°C)
Hard Clam	50°F (10°C)	68°F (20°C)
Oyster (any)	50°F (10°C)	77°F (25°C)

C. Turbidity

Turbidity in the sea water is of concern because turbidity adversely affects shellfish activity and turbidity reduces ultraviolet light penetration in the treatment of sea water.

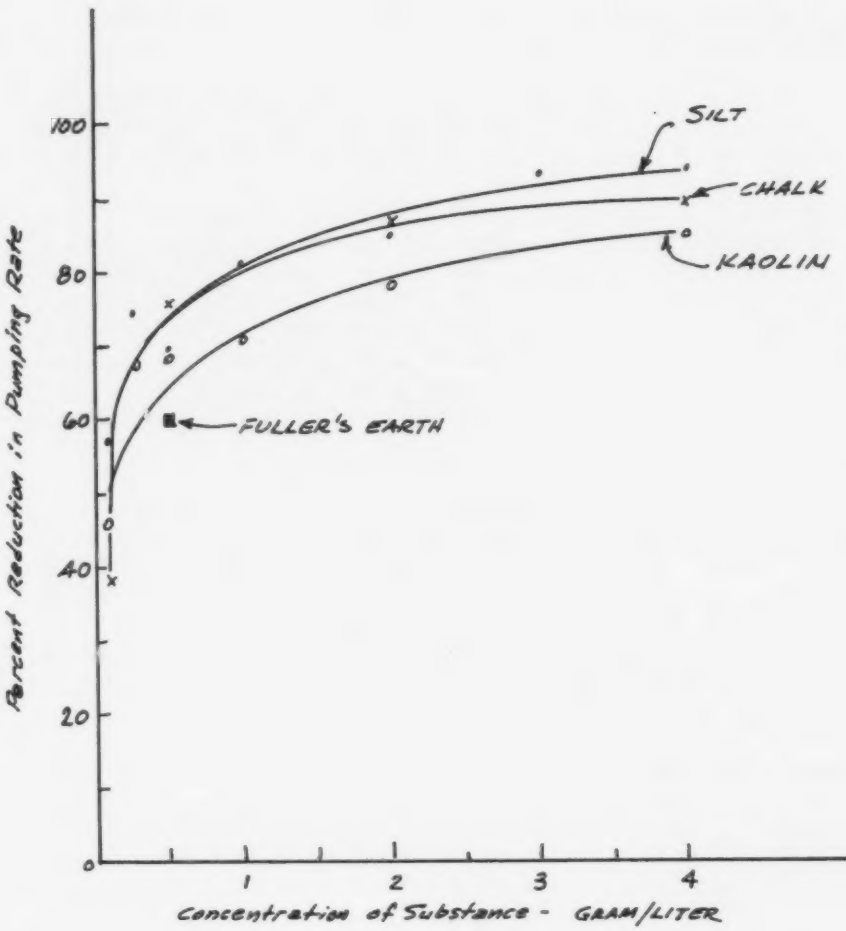
1. Affect on Shellfish. Loosanoff (1961) demonstrated that the pumping rate of 4 year old eastern oysters was markedly reduced even by rather low turbidities. This is shown in Figure A4 where it can be seen that at 0.1 gram/liter of kaolin, silt or chalk, reduced pumping rate to 38-57% of normal.

Loosanoff and Engle (1942 and 1947) described how different concentrations of different size plankton (which may be considered as turbidity) affect water pumping and shell movement of oysters. They postulated that in heavy plankton concentrations, the oysters were preoccupied with forming pseudofeces and therefore there was a minimal feeding. They found the following concentrations of microorganisms were sufficient to clog the gills:

Table A3. ALGAE AND GILL CLOGGING OF OYSTERS

Species	Concentration
Chlorella	2,000,000/milliliter
Nitzchia	70,000/milliliter
Euglena	3,000/milliliter

What turbidity units these figures represent is unknown but the conclusion is that algae blooms are undesirable.



After Loosanoff (1961)

FIGURE A 4

EFFECT OF TURBIDITY ON PUMPING RATE OF OYSTERS

JAN., 1965 NERC, FOA, SBF

Rice and Smith (1938) showed that filtering rates of Nitzchia closterium by the hard clam were reduced with the addition of 5 grams/liter of silt. It was concluded that since there was a great deal of silt-laden mucus released in the water whenever silt was added, that feeding (ingestion of Nitzchia cells) was reduced.

2. Affect on UV Treatment. Kelly (1961b) working with 20 turbidity units in sea water showed no serious interference with bacterial treatment of sea water by ultraviolet light.

Fresnell and Cummins (1965) correlated bacterial reduction in sea water with various turbidities for different types of UV units. Small commercial UV units, sized for flows of about 16 liters per minute, reduced coliform bacteria from 790 MPN/100ml to 0.2 MPN/100ml with a turbidity of 130 J.T.U. A Purdy Unit reduced coliform from 790 - 1300 MPN/100ml to 0.45 - 13 MPN/100ml with turbidity in the range of 100 - 130 J.T.U. and flows of 90 - 114 liters/minute (24 - 30 gallons/minute).

3. Conclusion. The governing factor in determining the maximum allowable turbidity in sea water is the detrimental effect of turbidity on shellfish activity. One hundred milligrams per liter (0.1 gms/liter or 100 turbidity units) were sufficient to cause a marked reduction in oysters' activity according to Figure A4. It is recommended that the maximum turbidity allowed in the process sea water be 20 J.T.U.

D. Hydrogen Ion Concentration (pH)

Hydrogen ion concentration has been related to biological activity of shellfish. Experiments with low pH has shown lowered activity.

1. The natural pH of sea water is in the range of 7.5-8.4.

2. Losanoff and Tommers (1947) showed reduced pumping rates of eastern oysters with reduced pH as follows:

Table A4. EFFECT OF pH ON PUMPING RATE OF C. VIRGINICA

pH	Remarks
7.75	Norman pumping.
6.75 - 7.0	Vigorous pumping for several hours, then reduction.
6.5	Decrease in pumping and less shell open time.
4.25	10% of normal pumping.

3. Galtsoff (1964) showed a detrimental affect of lowered pH with respect to oxygen uptake. The results are given in Table A5.

Table A5. EFFECT OF pH ON OXYGEN UPTAKE AT 25°C ON C. VIRGINICA

pH	Percent of Oxygen Consumption
8.1	98
6.7	65
5.8	20
5.4	8

4. Conclusion. A minimum pH of 7 and a maximum of 8.4 are recommended for the sea water, since deviations from this range may cause reduced shellfish activity.

E. Salinity

Salinity influences effectiveness of depuration because shellfish activity has been shown to be directly related to it. Extremes of this environmental factor have been shown to kill shellfish, as well as cause a necessary acclimatization to take place. Time taken by shellfish to

acclimatize to the stress of a change in salinity is time lost in depuration. These conclusions are based on the following:

1. Soft Clam. Pilot plant studies conducted by Sea and Shore Fisheries, State of Maine, (Goggins et al, 1964) indicate that soft clams, taken from waters of low salinity (18 o/oo) require 12 hours to overcome the stresses of acclimatization when they are placed in higher salinities (30 o/oo).

Matthiessen (1960) showed that for soft clams whose natural habitat was 18-30 o/oo, pumping rate is affected even at 15.5 o/oo. See Figure 5.

2. Oyster. Loosanoff (1952) found that C. virginica adapted to 27 o/oo was affected as follows:

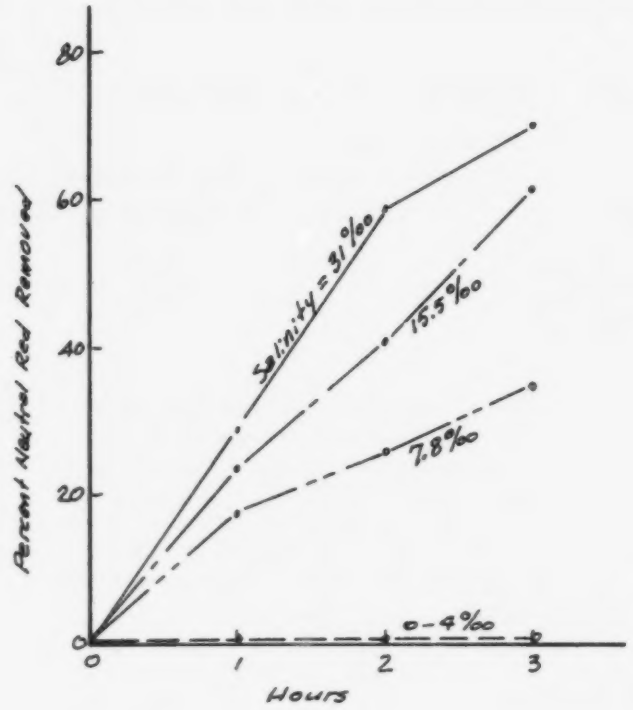
Table A6. ACCLIMATIZATION TO LOW SALINITIES BY C. VIRGINICA

Oysters acclimatized at 27 o/oo

New Salinity	Percent of Normal Pumping (for 6 hours)
20 o/oo	76%
15 o/oo	11%
10 o/oo	9%
5 o/oo	0.4%

Pyle and Dawson (1950) found that salinities differing from week to week by 11 o/oo, and during a tidal cycle by 4.8 o/oo, did not interfere with the well-being of the Florida oyster (C. virginica). Medcof (1944) stated that oysters in Canadian waters starve at salinities less than 20 o/oo.

Hopkins (1936) found almost no pumping in O. gigas at a salinity of 10.5 o/oo but some at 13 o/oo. He found that adaption to a lower or higher salinity may never be complete as salinity departs further from the accustomed salinity. Also, he found that oysters took longer to adapt to salinities of less than 20 o/oo.



After Mathiesson (1960)

NOTE: Normal Habitat of these Soft Clams had Salinity Range 18-30 ‰.

FIGURE A-5

RELATIVE PUMPING RATES OF SOFT CLAMS AT DIFFERENT SALINITIES

JAN., 1965 NERS, FOA, SAF

3. General. Wells (1961) described the salinity death points for C. virginica to be 7 o/oo and for M. mercenaria to be zero o/oo. Chanley (1948) demonstrated minimum survival salinities for the following juvenile bivalves:

Table A7. MINIMUM SURVIVAL SALINITIES -- o/oo

Species	After Direct Transfer From 27 o/oo	After Conditioning at Intermediate Salinities
Mya arenaria	2.5 - 10	2.5
M. mercenaria	12.5 - 15	12.5
C. virginica	5 - 7.5	2.5 - 5.0

4. Conclusion. Even though shellfish can adapt somewhat to changes in salinity, it has been shown that activity is affected and that acclimatization takes time. Therefore it appears reasonable that the salinity of the depuration water should be in the range of 100±20% (percent) of the value of salinity of the harvest area. Shellfish harvested from areas of significantly different salinities may require different sea water systems in one depuration plant.

F. Oxygen and Flow Rate

Oxygen depletion through respiration of shellfish is directly related to flow rates of sea water. The greater the flow rate the more oxygen is provided to the shellfish, and therefore the lower the depletion.

1. Soft Clam. Van Dam (1935) showed that oxygen utilization of soft clams was independent of oxygen concentration down to about 2 cc/liter (2.8 mg/l). He also showed that the oxygen requirement (utilization) was much higher for the first 2-3 hours that clams were exposed to water after having been out of water for a period of a low tide.

Experiments by Goggins, Hurst, Mooney (1964) conducted at Boothbay Harbor, Maine, showed that at flow rates below one liter per minute per bushel of soft clams, 90% depletion of oxygen occurred with temperatures ranging from 16°C - 20°C. On increasing flow to 1.5 to 2.5 liters per minute per bushel 50% of the oxygen was depleted and bacterial reduction was greater than for the lower flows.

2. Oyster. Pedersen (1947) arrived at the following temperature-oxygen uptake relationships for O. edulis:

Table A8. OXYGEN UPTAKE OF OYSTERS

Temp.°C	cc/100 gms Oyster/24 hrs.	mg/100 gms Oyster/hour*
0	Hardly measurable	
5	2	0.12
10	5	0.30
15	10	0.60
20	20	1.20
25	30	1.80

*(cc/100gm oyster/24 hrs)(1.43 mgO_2/cc)($\frac{1}{24}$) = mg/100gm oyster/hr:

Weight of oyster in terms of total weight (shell & soft parts).

This data is plotted in Figure A6. Since it appears that the increase in oxygen uptake parallels that of pumping rate (Figure A2), the oxygen uptake was extended to about 30°C, where an uptake of about 2.4 mg of oxygen per 100 gm oysters per hour occurs.

Wood (1961) presented information on oxygen uptake for O. edulis from which the following was derived:

Table A9. OXYGEN UPTAKE OF OYSTERS

Temp.°C	Oxygen Uptake mg/Oyster/hour
1.1	0.06
5.2	0.14
10.6	0.5
15.0	1.0

These data are also plotted in Figure A6 for comparison with Pedersen's data.

Galtsoff and Whipple (1931) concluded that oxygen consumption by C. virginica is not influenced by oxygen tension when the amount is greater than 2.5 cc/liter (3.5 mg/liter), and that below this oxygen consumption was affected. Hammen (1962) presenting

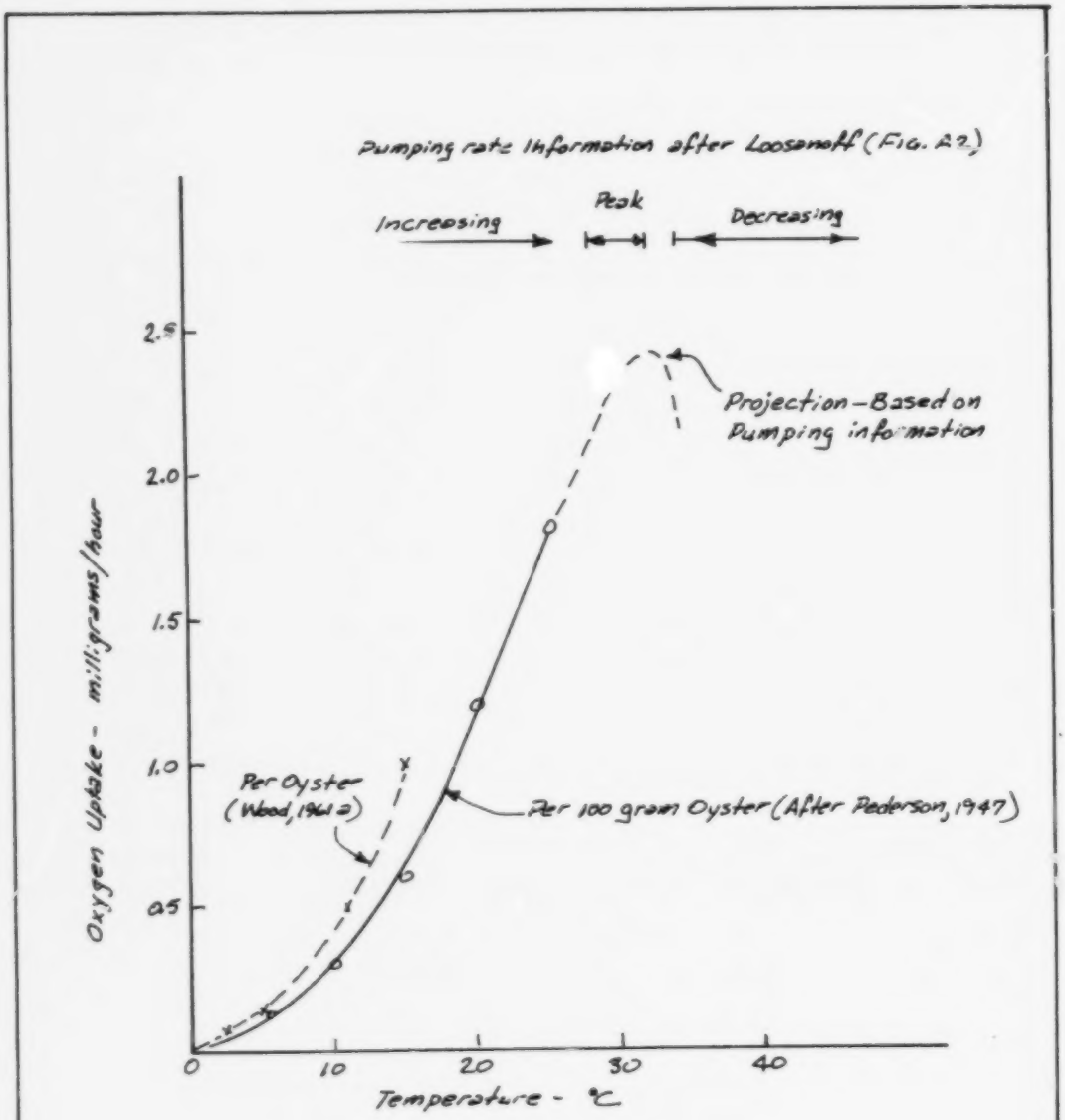


FIGURE A6
OXYGEN UPTAKE VS. TEMPERATURE FOR OSTREA EDULIS

Oct., 1969 NERC, PDA, SW

unpublished data by Galtsoff for C. virginica, described an oxygen consumption of 0.039 ± 0.01 ml/gm total body weight/hour for oysters of mean total weight of 103.5 gms. On comparing this with Pedersen's 100 gram oysters (O. edulis), the oxygen consumption for C. virginica would be 3.9 ± 1 ml/oyster/hour, or somewhat higher than O. edulis. Mitchell (1914) found for a temperature range of 19-28°C that oysters consumed 0.7 to 3.4 mg of oxygen/100 gms total weight/hour, and that the uptake-temperature relation was almost a straight line.

3. Calculations and Conclusions

a. Minimum flow of water. Factors used as follows: Oxygen uptake - 1.0 mg/shellfish/hour for temperature range 15°-20°C (approximately equivalent to Wood's, Pedersen's and Mitchell's oyster data); saturation value for oxygen at 15°C = 8.1 mg/l (at a salinity of 36 o/oo); assume 500 shellfish/bushel.

$$\text{mg/shellfish/hr} = \frac{(\text{mg/l})\ (\text{liters/min/bushel})\ \frac{(60\ \text{min})}{\text{hr}}}{500\ \text{shellfish/bushel}}$$

$$1.0 = (8.1)\ (Q)\ \frac{60}{500} \quad ; \quad Q = \text{liters per minute per bushel}$$

$$Q = \frac{1.0 \times 500}{60 \times 8.1} = 1.04\ \text{lpm/bushel}$$

This assumes that all oxygen is used up. It the water was at 50% of saturation 2.08 lpm/bushel would be required; but all the oxygen here would be used up also. To insure that shellfish at the farthest point downstream in a depuration tank receive an adequate supply of oxygen it is recommended that 4 lpm/bushel (or about one gallon per minute/bushel) be used.

b. Minimum oxygen concentration. If the flow rate is maintained at 4 lpm and oxygen consumption is about 2.4 mg/shellfish/hour (see Figure A5) then the minimum oxygen concentration is as follows:

$$(2.4) = \left(\frac{\text{mg}}{\text{l}}\right)\ (4\ \text{lpm/bush})\ \frac{60}{500}$$

$$\text{mg/l} = \frac{2.4 \times 500}{4 \times 60} = 5\ \text{mg/l}$$

G. Metals

Metals in the process sea water should not exceed that normally found in sea water. The reasons are (1) that shellfish concentrate metals (especially bivalent ones) in their tissue by means of adsorption of metals to the mucus creating a public health problem and (2) metals inhibit pumping.

1. McFarren (1961) reported a zinc concentration in oysters from Chesapeake Bay at 4000 mg/kg of body weight and copper as high as 520 mg/kg in oysters from West Neck, Long Island, New York. These values represent about 130 mg zinc and about 17 mg copper per oyster. Galtsoff (1932) reported that a green oyster contains about 2.5 mg of copper.

Zinc salts cause emesis in humans at a concentration range in water of 675 to 2,280[1] mg/liter.

Polyvalent ions such as Al^{+++}, Fe^{++}, Hg^{++} and Mn^{++} are caught and accumulated in the mucus of the oyster according to Korringa (1952).

Sea water containing zinc, copper, or other metals at concentrations above that usually found in sea water must not be used for depuration.

2. Iron in low concentrations (1 ppm as $FeCl_3$) reduces transmittivity of ultraviolet light from 90% to 5% according to Summer (1962).

H. Pesticides

1. Butler, Lowe and Wilson (1963) presented the following information on accumulation of DDT by shellfish:

---

[1]PHS - 1962 - "Drinking Water Standards."

Table A10. UPTAKE OF PESTICIDES BY SHELLFISH

Species	Amt. of DDT In Environment	Exposure Time Days	Amt. in Tissues
East. Oyster	10 ppb	7	151 ppm
East. Oyster	1 ppb	35	17 ppm
Pacific Oyster	1 ppb	7	20 ppm
Hard Clam	1 ppb	7	3-9 ppm

This information indicates a sizeable accumulation in a short time. They also presented the following as an indication of the slow elimination of DDT by shellfish when exposed to clean water:

Table A11. RETENTION OF PESTICIDES IN SHELLFISH

	Residual DDT ppm in Tissue						
	Days in Clean Sea Water						
Species	0	10	20	30	50	70	90
Hard Clam	3.5	0.88	0.161				
Oysters (Eastern)	151	128		119	44	24	6.5

2. Conclusions. The above information leads to two conclusions. The first is that shellfish which have been exposed to waters in harvest areas which have received runoff from agricultural land, may contain pesticides at a level unacceptable to the Federal Food and Drug Administration. The depuration process probably will not eliminate these pesticides to a satisfactory level. Secondly, the sea water used for depuration must have very low amounts of pesticides because in a matter of days, the levels in tissue of shellfish become high. The amount allowed in the water is predicated on the maximum amount allowed in the tissue of the shellfish to be depurated.

I. Marine Toxins

McFarren, et al (1960) relates that there have been no feasible commercial practices for detoxifying shellfish from Paralytic Shellfish Poison (PSP), including transplanting. It is not known whether ciguatera like poison can be depurated from shellfish.

In those geographical areas of concern for any type of natural marine toxin, the waters cannot be used for depuration, nor can the shellfish in those waters be depurated by the conventional methods, unless careful monitoring is carried out.

McFarren, et al (1960) reports the following on PSP: if a large number of Gonyaulax (the causative plankton) is present in the water, the toxicity of bivalves may rise in a few days to a dangerous level; besides the stomach as a site of accumulation of the poison, the poison settles in the siphon of the butter clam, and the gills of soft clams as a secondary site.

J. Further Notes on Feeding and Activity of Mollusks

1. Time of Feeding. Loosanoff and Nomejko (1946) found that for C. virginica, food passes thru the entire intestinal tract in 80-150 minutes at 20°C, and that oysters fed as much at night as during the day. They found that the shell was open for the same duration whether the tide was at ebb or at flood. Galtsoff (1928b) found that an oyster keeps open about 17 hrs. and 7 minutes per 24 hours and that there is no correlation between opening and closing of shell versus time of day. However, Bennett (1954) described for Mercenaria mercenaria a persistent diurnal rhythm of opening and closing of the shell with a maximum in the morning and a minimum in the afternoon. Also this phase relationship to time of day could be shifted by exposing these clams to 100 foot candles of light by night and to darkness by day for five consecutive 24 hour periods.

2. Type of Food. Cerruti (1941) listed the stomach contents of O. edulis as follows: organic detritus, diatoms, flagellates, larvae of annelids, sand, silt, spicule of sponges, larvae of molluscs, eggs and gastrulae of marine invertebrates, plant fibers and pollen grains.

Coe (1947) described nutrition of bivalve molluscs as being provided by intracellular digestion of detritus of disintegrated cells of marine plants and animals, very small diatoms, bacteria, minute flagellates and other protozoa and gametes of invertebrates. Also he said the gut usually contains a large amount of sand and other undigestible particles.

3. Stimulants. Collier, et al (1953) reported that the activity of C. virginica on the Gulf Coast could be correlated with the presence of a carbohydrate-like chemical in the waters. Galtsoff (1964) did not concur with their conclusions, however, after experiments at Cape Cod, Massachusetts.

4. General. Chipman (1947) said that, in general, temperature, salinity, currents, availability of food, type of food, alkalinity, turbidity -- all -- play an important role in the quantity of water passing thru the gills and in the frequency of opening and closing of the shells.

In the Commonwealth of Massachusetts Report (1947) it is stated that "Studies ... have shown that (soft) clams require the presence of food in the process water in order for them to purge themselves."

II. Derivation of Bacterial Levels Correlated with Time for Depuration

A. Experimental Depuration Runs

Reduction of coliform organisms in shellfish during experimental and pilot plant studies have been observed for the soft clam, the hard clam and the oyster. The results of more recent works are shown in Figures A7 thru A14. These results show that shellfish, when placed in relatively clean waters or in treated waters, purge themselves of coliform bacteria.

Coliform bacteria in shellfish are presumably in the mantle fluid, on the surfaces of the soft parts and in the alimentary canal. For example Boury and Borde (1964) found coliform bacteria in the mantle, gills, visceral mass, mucus and mantle fluid in mussels. The coliform may also be in other cavities to some degree.

The coliform in the mantle cavity are probably the first to be emitted since the mantle fluid is quickly replaced by the surrounding clean sea water. The coliform on the surfaces of the soft parts may be slowly cleaned away, mechanically, thru ciliary action and the production of pseudofeces. Coliforms in the gut may be destroyed in the alimentary canal or else mechanically excreted in the feces thru normal intestinal action.

Table A12 shows the levels to which coliform values are reduced after 24 and 48 hours of depuration. The data are taken from Figures A7 thru A14.

Based on the information in Table A12, the conservative percent coliform remaining which can reasonably be expected for the various shellfish is as shown in Table A13:

Table A13. GENERAL LEVEL OF COLIFORMS REMAINING

Shellfish	Percent Coliform Remaining After 24 hours	After 48 hours
Soft Clam	25	10
Hard Clam	5	2
Oyster	5	2

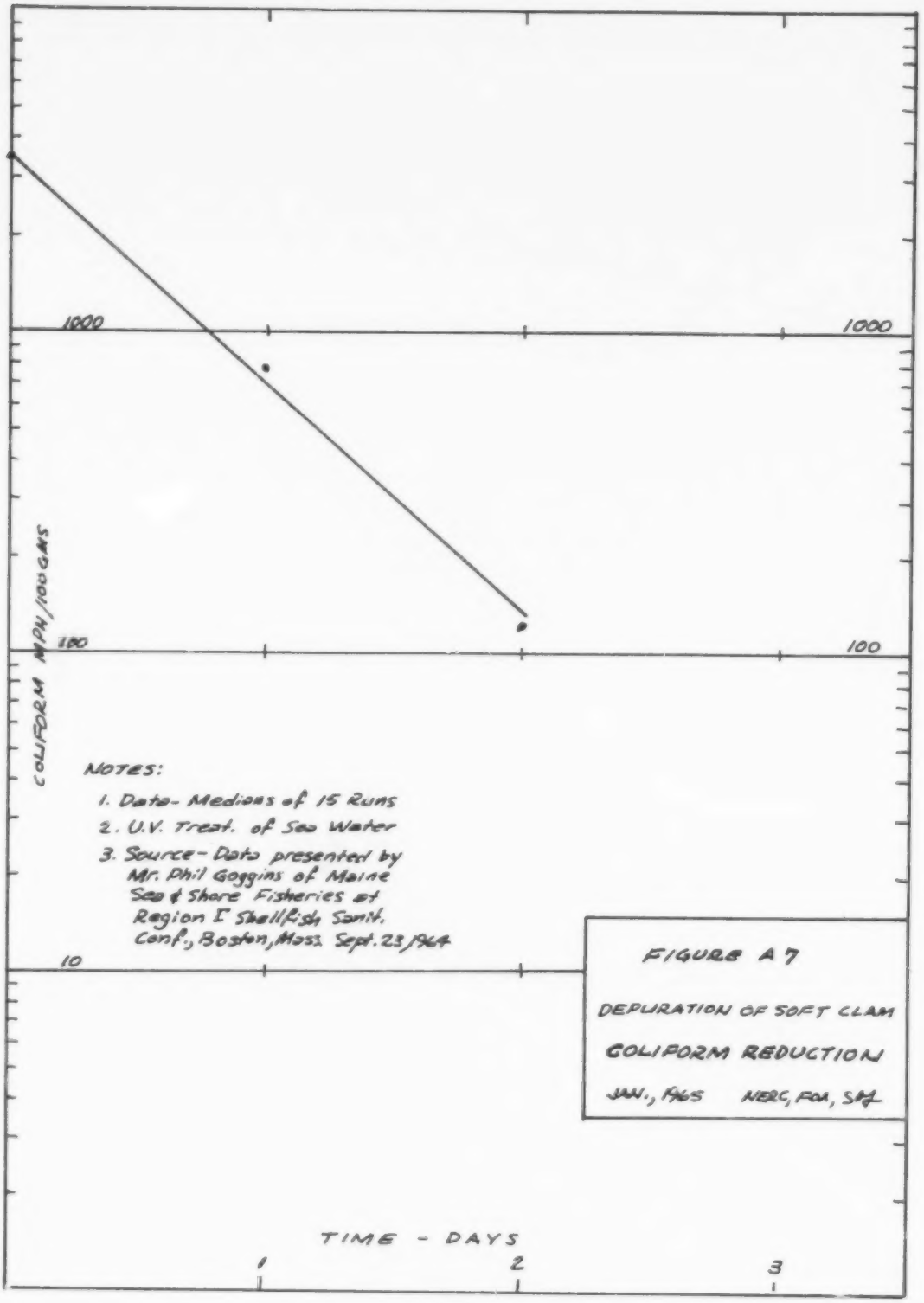


FIGURE A 7

DEPURATION OF SOFT CLAM

COLIFORM REDUCTION

JAN., 1965 NERC, FOA, SAL

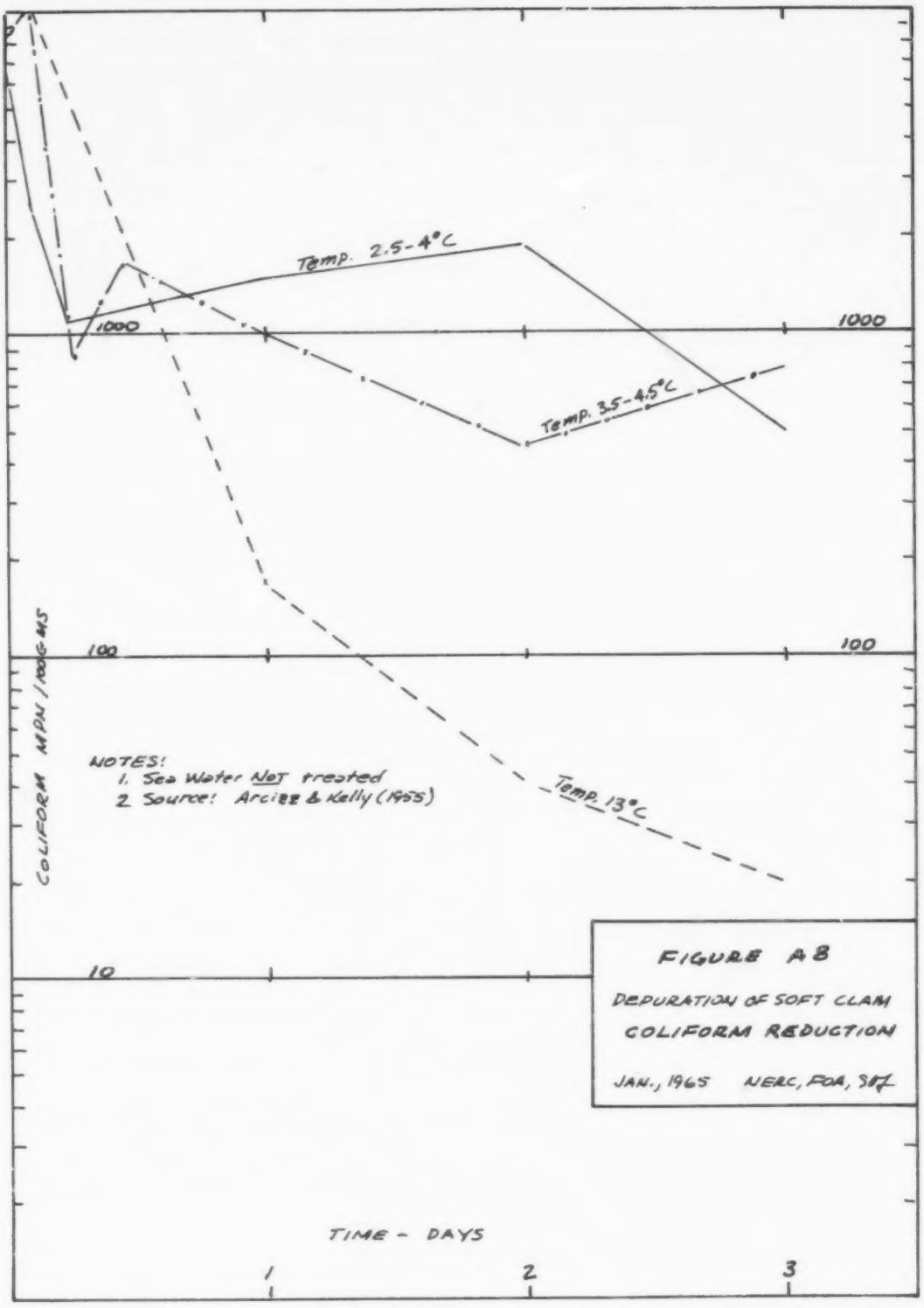


FIGURE A8

DEPURATION OF SOFT CLAM
COLIFORM REDUCTION

JAN., 1965 NERC, FOA, SEF

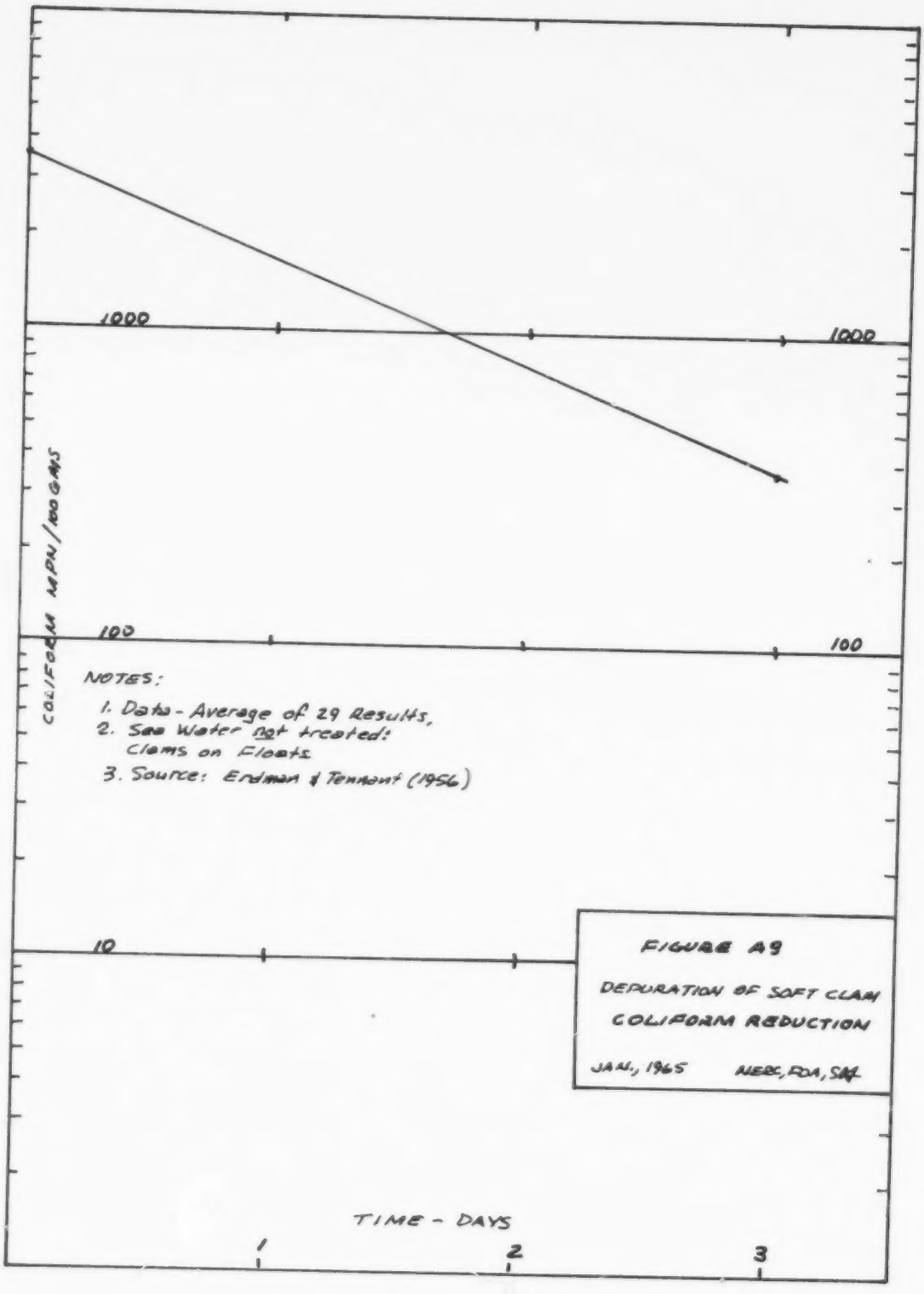


FIGURE A9

DEPURATION OF SOFT CLAM
COLIFORM REDUCTION

JAN., 1965 NERC, FDA, SAF.

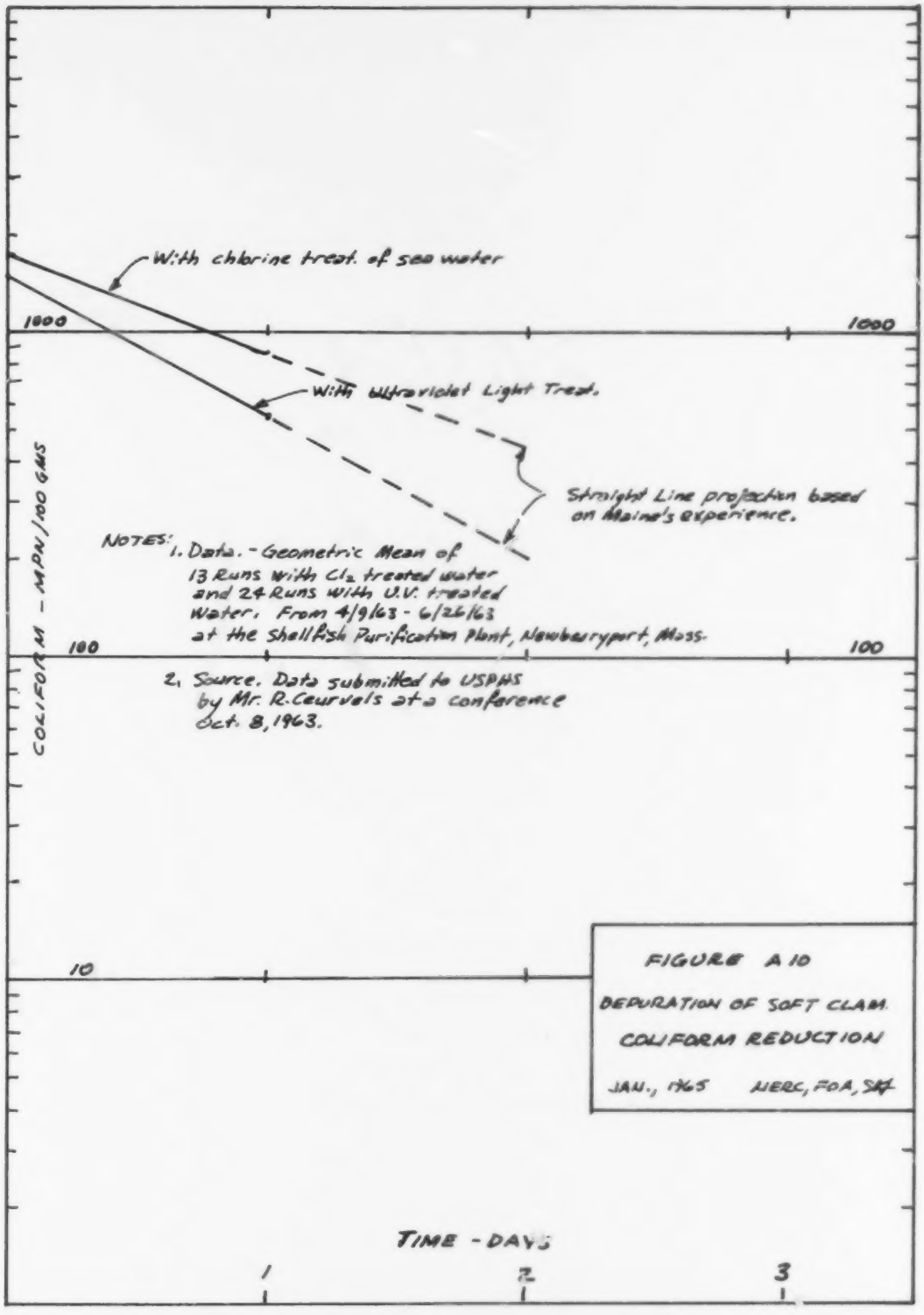
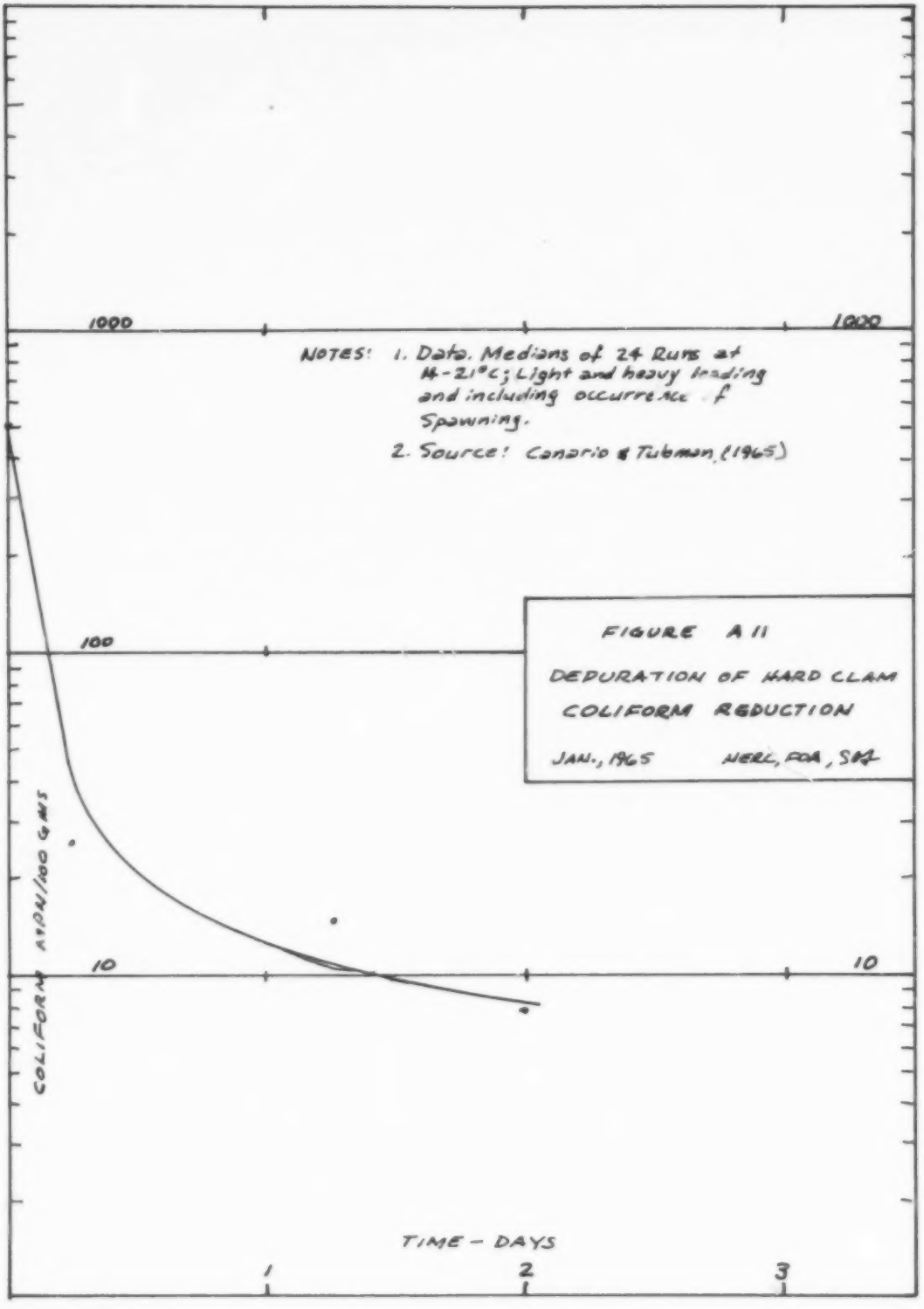
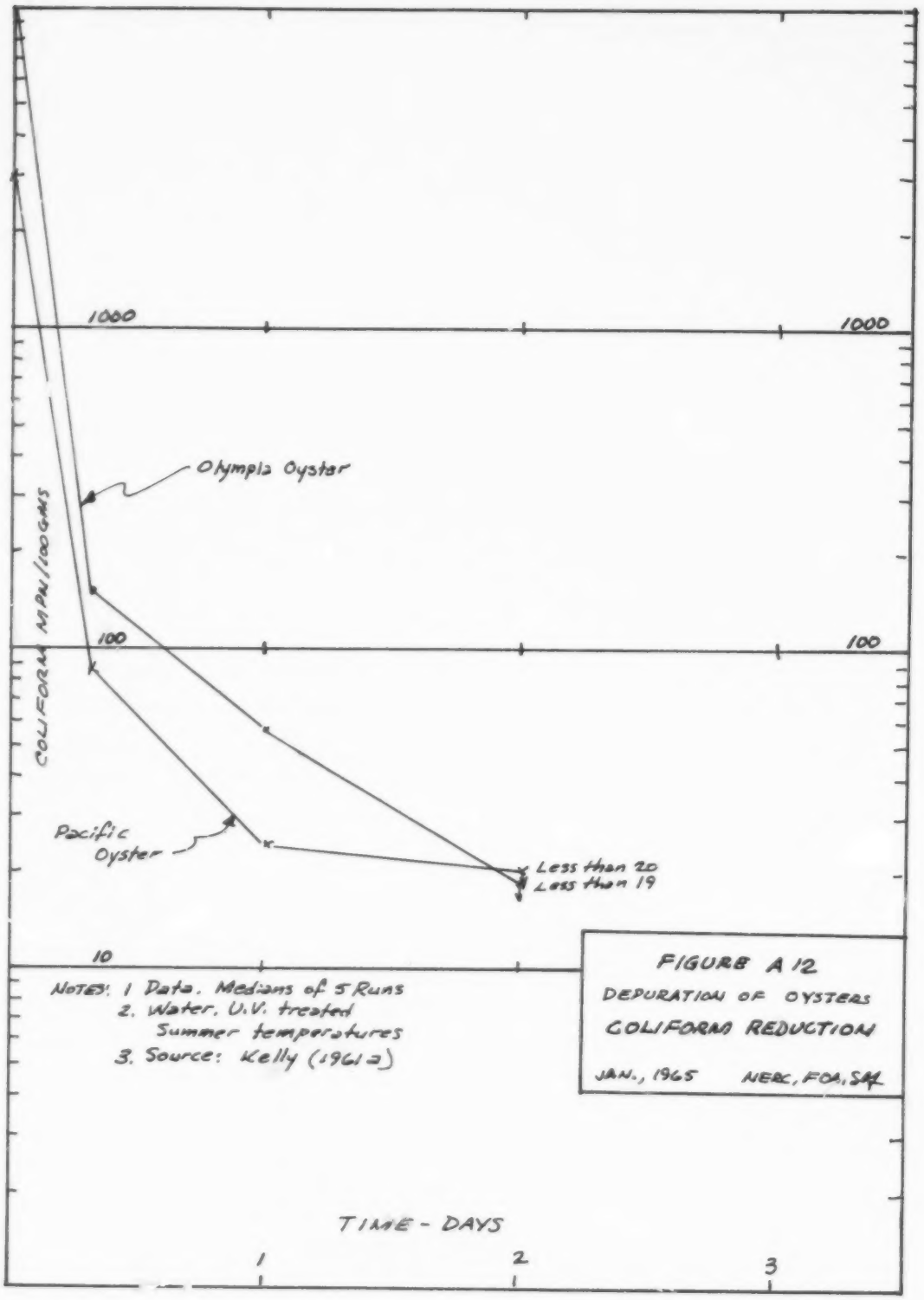

With chlorine treat. of sea water
With ultraviolet Light Treat.
Straight Line projection based on Maine's experience.
NOTES:
1. Data. - Geometric Mean of 13 Runs With Cl_2 treated water and 24 Runs With U.V. treated Water. From 4/9/63 - 6/26/63 at the Shellfish Purification Plant, Newburyport, Mass.
2. Source. Data submitted to USPHS by Mr. R. Ceurvels at a conference Oct. 8, 1963.
COLIFORM - MPN/100 GMS
1000
100
10
TIME - DAYS
1
2
3

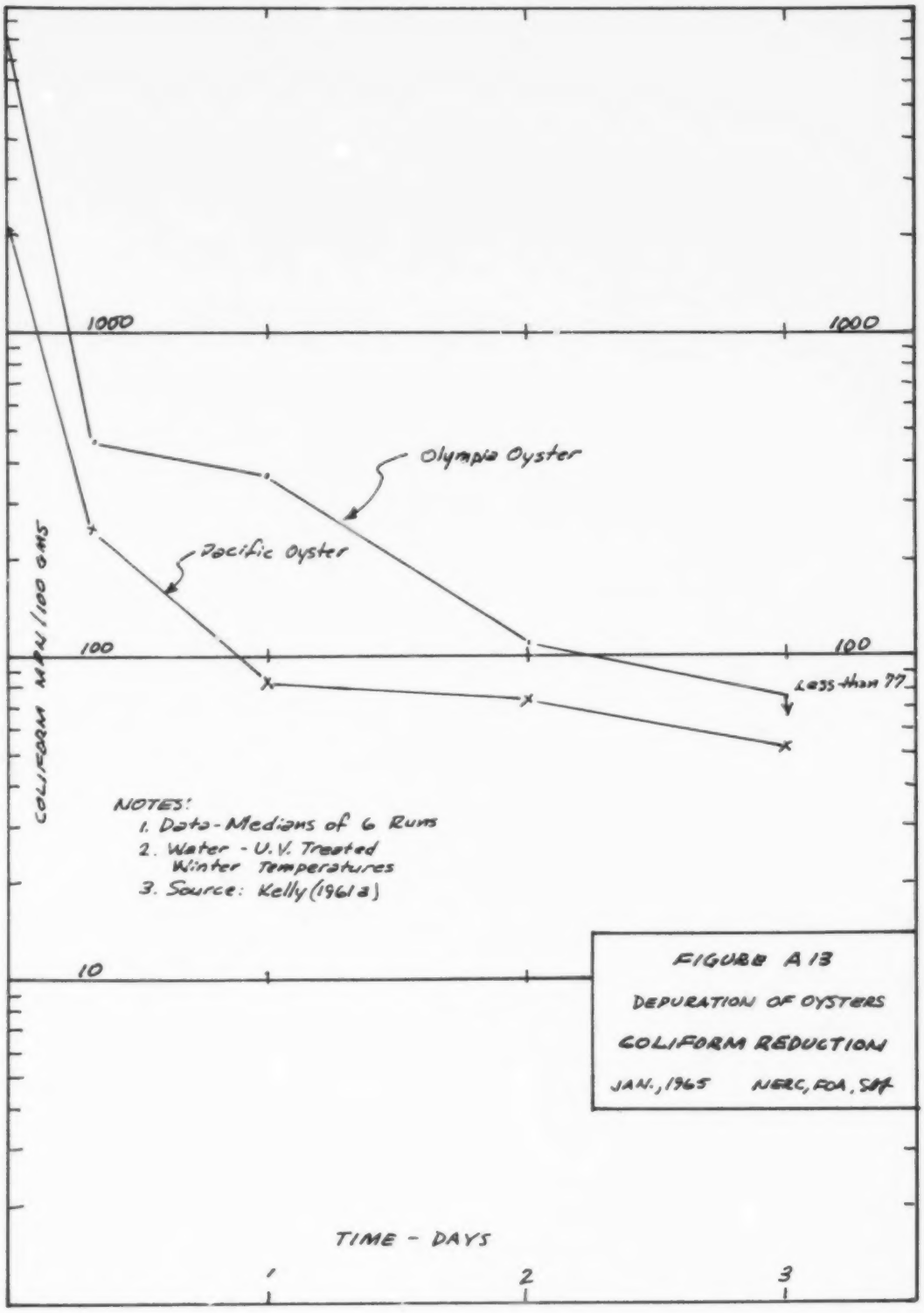
FIGURE A 10

DEPURATION OF SOFT CLAM. COLIFORM REDUCTION

JAN., 1965 NERC, FDA, SKF


1000
1000
NOTES: 1. Data. Medians of 24 Runs at 14-21°C; Light and heavy loading and including occurrence of Spawning.
2. Source: Canario & Tubman (1965)
100
FIGURE A 11
DEPURATION OF HARD CLAM COLIFORM REDUCTION
JAN., 1965 NERL, FDA, SOJ
COLIFORM MPN/100 GMS
10
10
TIME - DAYS
1
2
3


1000
1000
COLIFORM MPN/100GMS
Olympia Oyster
100
100
Pacific
Oyster
Less than 20
Less than 19
10
NOTES: 1 Data. Medians of 5 Runs
2. Water. U.V. treated
Summer temperatures
3. Source: Kelly (1961a)
FIGURE A 12
DEPURATION OF OYSTERS
COLIFORM REDUCTION
JAN., 1965 NERC, FOA, SAF
TIME - DAYS
1
2
3


1000
1000
Olympia Oyster
Pacific Oyster
COLIFORM MPN/100 GMS
100
100
Less than 77
NOTES:
1. Data - Medians of 6 Runs
2. Water - U.V. Treated
Winter Temperatures
3. Source: Kelly (1961a)
10
FIGURE A 13
DEPURATION OF OYSTERS
COLIFORM REDUCTION
JAN., 1965 NERC, FDA, SAF
TIME - DAYS
1
2
3

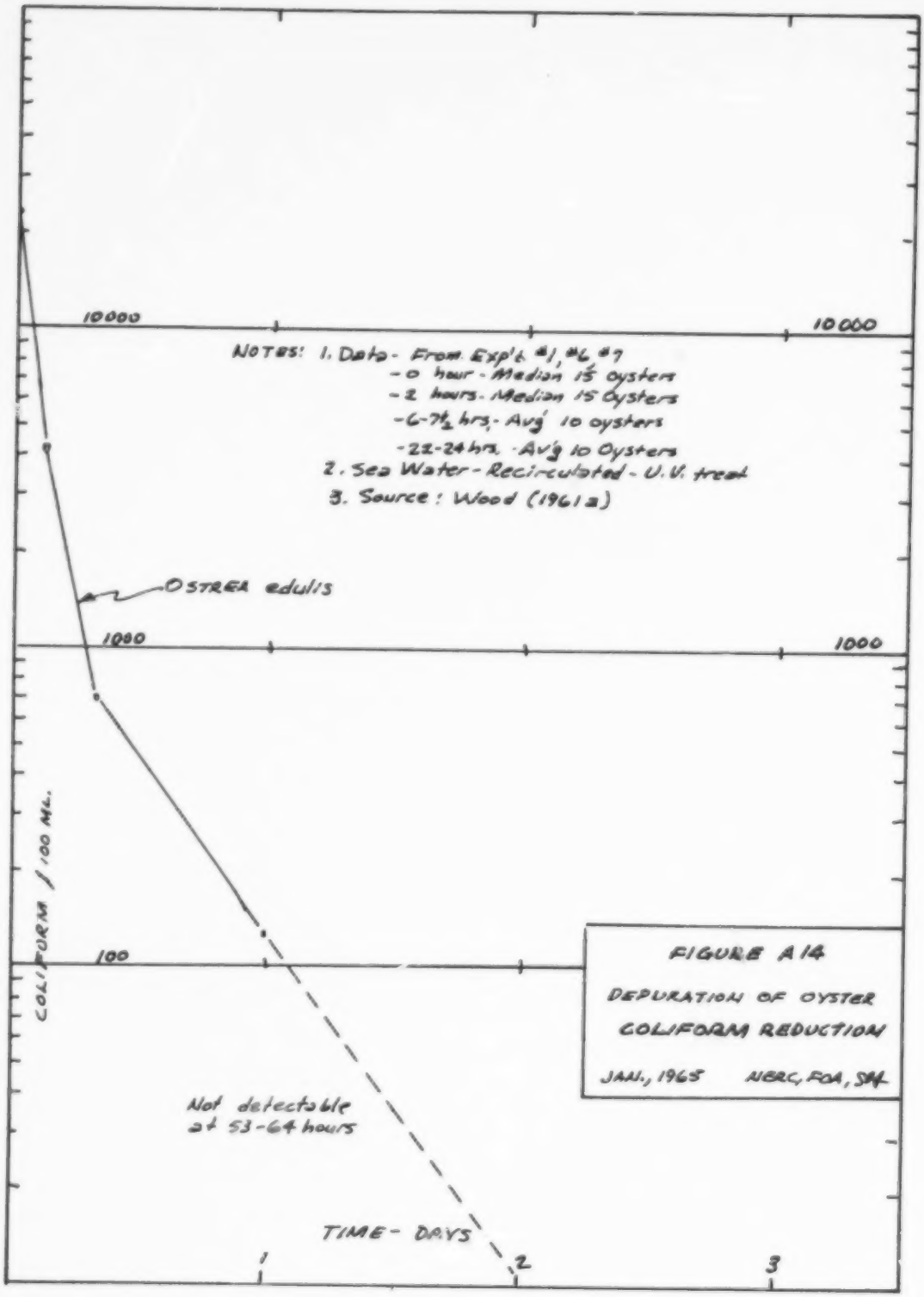

10000
10000
NOTES: 1. Data - From Expt's #1, #6 #7
- 0 hour - Median 15 oysters
- 2 hours - Median 15 Oysters
- 6-7½ hrs. - Avg 10 oysters
- 22-24 hrs. - Avg 10 Oysters
2. Sea Water - Recirculated - U.V. treat
3. Source: Wood (1961a)
OSTREA edulis
1000
1000
COLIFORM / 100 ML.
100
FIGURE A 14
DEPURATION OF OYSTER
COLIFORM REDUCTION
JAN., 1965 NERC, FOA, SHA
Not detectable
at 53-64 hours
TIME - DAYS
1
2
3

Table A12. RESULTS OF DEPURATION RUNS - COLIFORM ORGANISMS/100 GMS

Place	Shellfish	Initial Number	After 24 Hours No.	After 24 Hours % Remaining	After 48 Hours No.	After 48 Hours % Remaining	Water Treatment	References*
Maine	Soft Clam	3,500	700	20	120	3.4	UV, Var. Temp.	Goggins, et al (1964)
Woods Hole, Mass.	Soft Clam	10,000	1,000	10	450	4.5	None - 3.5°C	Arcisz & Kelly (1955)
Woods Hole, Mass.	Soft Clam	10,000	170	1.7	40	0.4	None - 13°C	" " "
Canada	Soft Clam	3,500	1,600	46	800	23	None - Floats - Var. Temp.	Erdman & Tennant (1956)
Newburyport, Mass.	Soft Clam	1,700	860	50	450	26	Chlorine	Ceurvels (1963)
Newburyport, Mass.	Soft Clam	1,500	550	36	200	13	UV	" "
Bristol, R.I.	Hard Clam	500	12	2.4	8	1.6	UV	Canario & Tubman (1965)
Purdy, Wash.**	Pacific Oyster	3,500	25	0.7	<20	<0.5	UV - Summer	Kelly (1961a)
	Pacific Oyster	2,100	84	4.0	74	3.5	UV - Winter	
	Olympia Oyster	15,000	55	0.4	<19	<0.1	UV - Summer	
	Olympia Oyster	8,300	360	4.4	120	1.4	UV - Winter	
Great Britain	Ostrea edulis	23,000	130	0.6	10	0.04	UV - Recirculated	Wood (1961a)

*See Charts A7 - A14.
**E. coli results.

B. Depuration of Salmonella

Figure A15 presents the result of depuration of the soft clam of Salmonella bacteria. It can be seen that relatively low levels of the Salmonella organism were attained in a day and that by the second day, in general, the presence of the organism was not demonstrable according to the technique. This Salmonella data will not be used in the ensuing analyses so as to maintain consistency of the analyses.

C. Growing Area Surveys and Laboratory Results

Several field surveys and laboratory works have been conducted to show relationships of coliform concentration in the water to the coliform concentration in the shellfish.

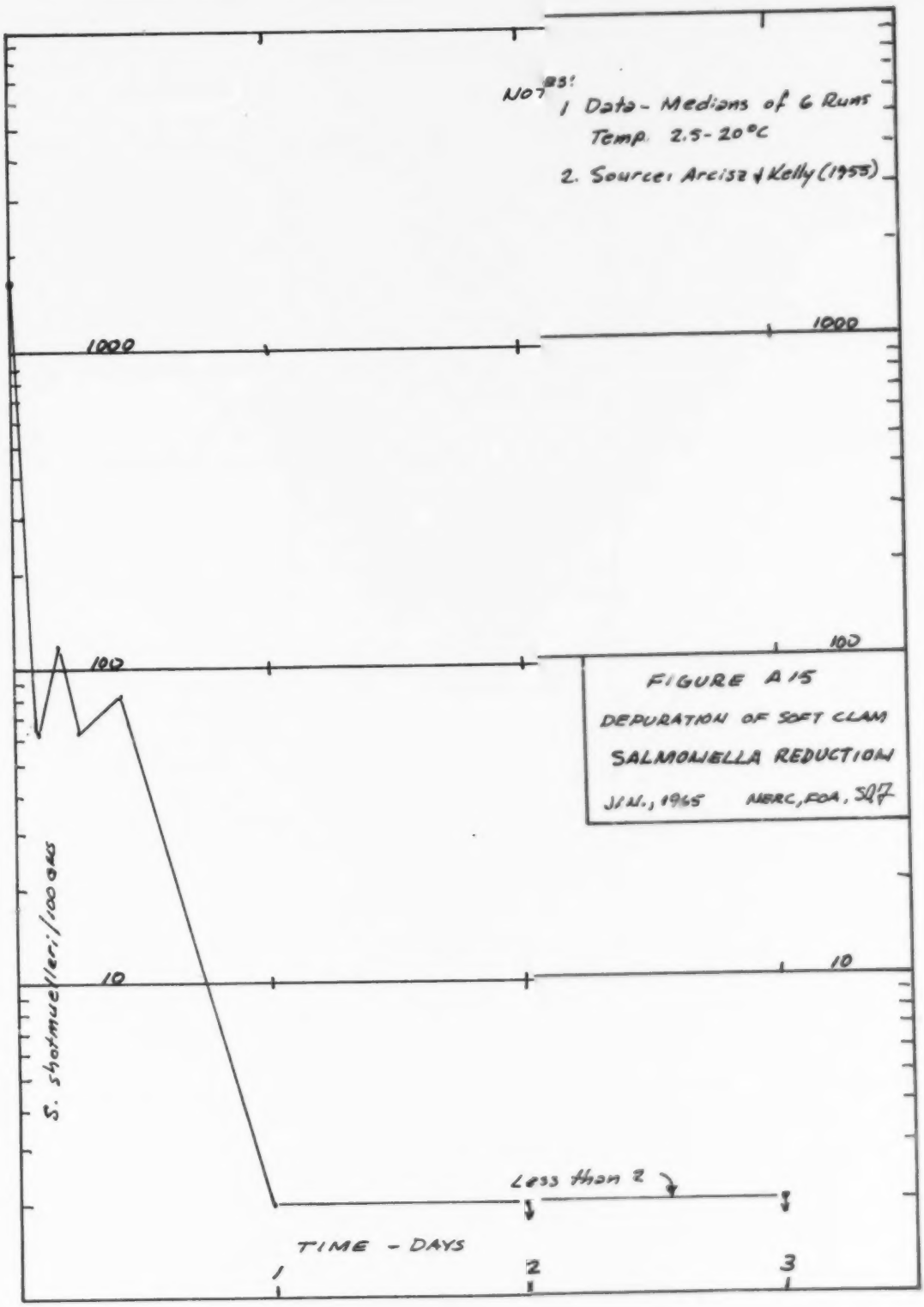
Figure A16 shows the results of a field survey conducted in Maryland on the soft clam. Figure A17 presents the results of the Hard Clam, Raritan Bay Study of 1941. Figures A18 and A19 show results of data on the eastern oyster for growing areas.

Laboratory experiments conducted specifically to show this concentration phenomenon were conducted at Woods Hole, Massachusetts, on the soft and hard clams and the eastern oyster and at Pensacola, Florida, on oysters by Kelly (1956, 1960). Results of these experiments are shown in Figures A20 thru A23.

The remarkable feature of these curves, in comparing the field work with the laboratory work, is the flatness of the lines for the field work and the relative steepness of the lines for the laboratory work. The exact biological significance of this may relate to concentration in the mantle fluid but this is not substantiated.

However, there is some order to the relation of the level of coliform concentration in shellfish to that of the water.

Table A14 presents the data used to plot the growing area curves for Maryland soft clams and Gulf Coast oyster. The regression lines in Figure A18 were derived from paired values which are not shown. The correlation coefficients for the two lines were 0.63 for the summer data and 0.76 for the winter data, both of which are significant at $P = 0.05$. Table A14 also gives the corresponding shellfish values for water a MPN of 70 from these growing area charts and also values from Figure A20 thru A23 which represent the controlled experiments. Since the E.C. positive test is becoming of interest, some results for the growing area surveys are also given.


NOTES:
1. Data - Medians of 6 Runs
Temp. 2.5 - 20°C
2. Source: Arcisz & Kelly (1955)
1000
100
10
S. shotmueller/100 GMS
Less than 2
TIME - DAYS
1
2
3
FIGURE A 15
DEPURATION OF SOFT CLAM
SALMONELLA REDUCTION
J.N., 1965 NBRC, FDA, SQF

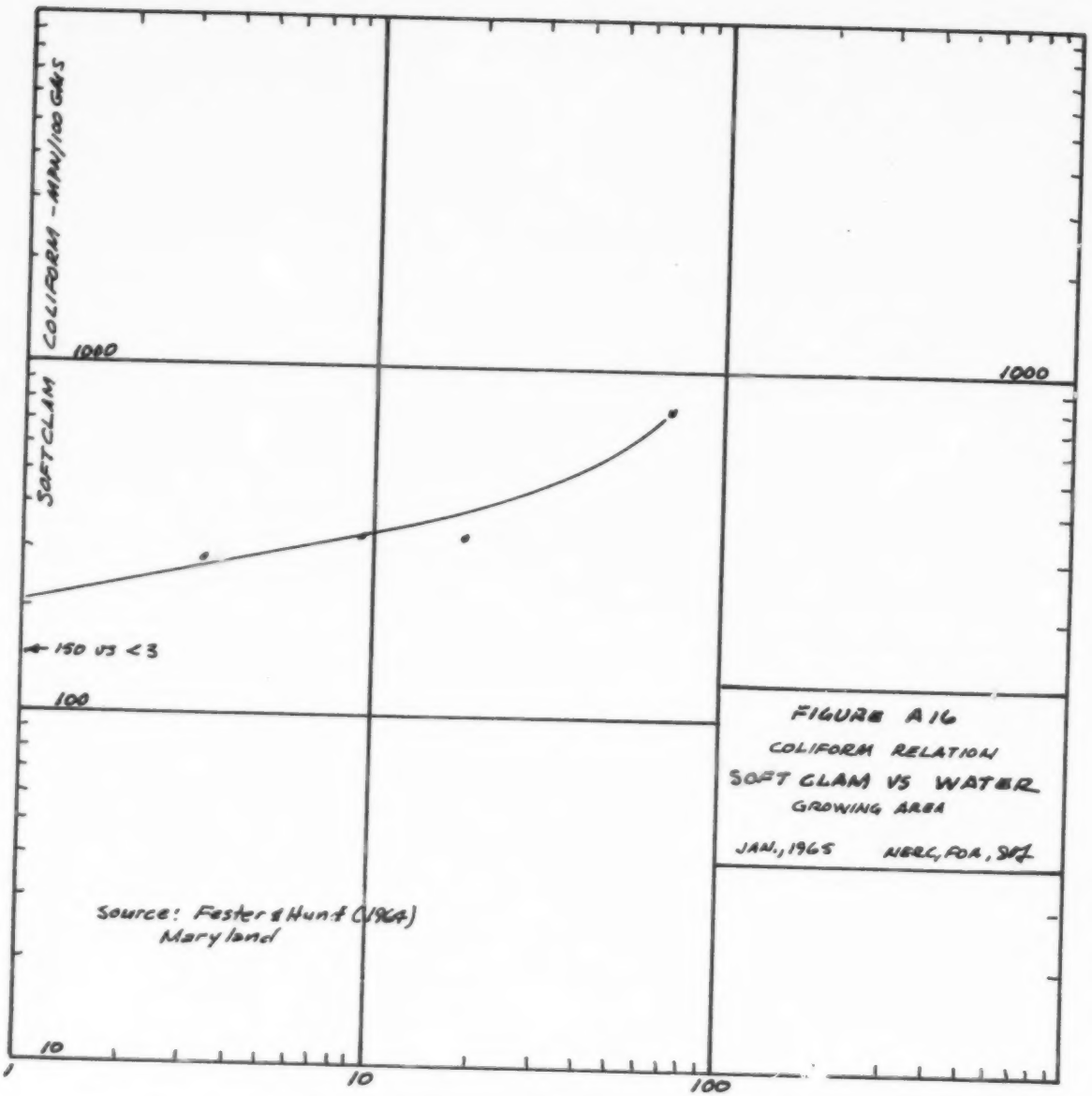
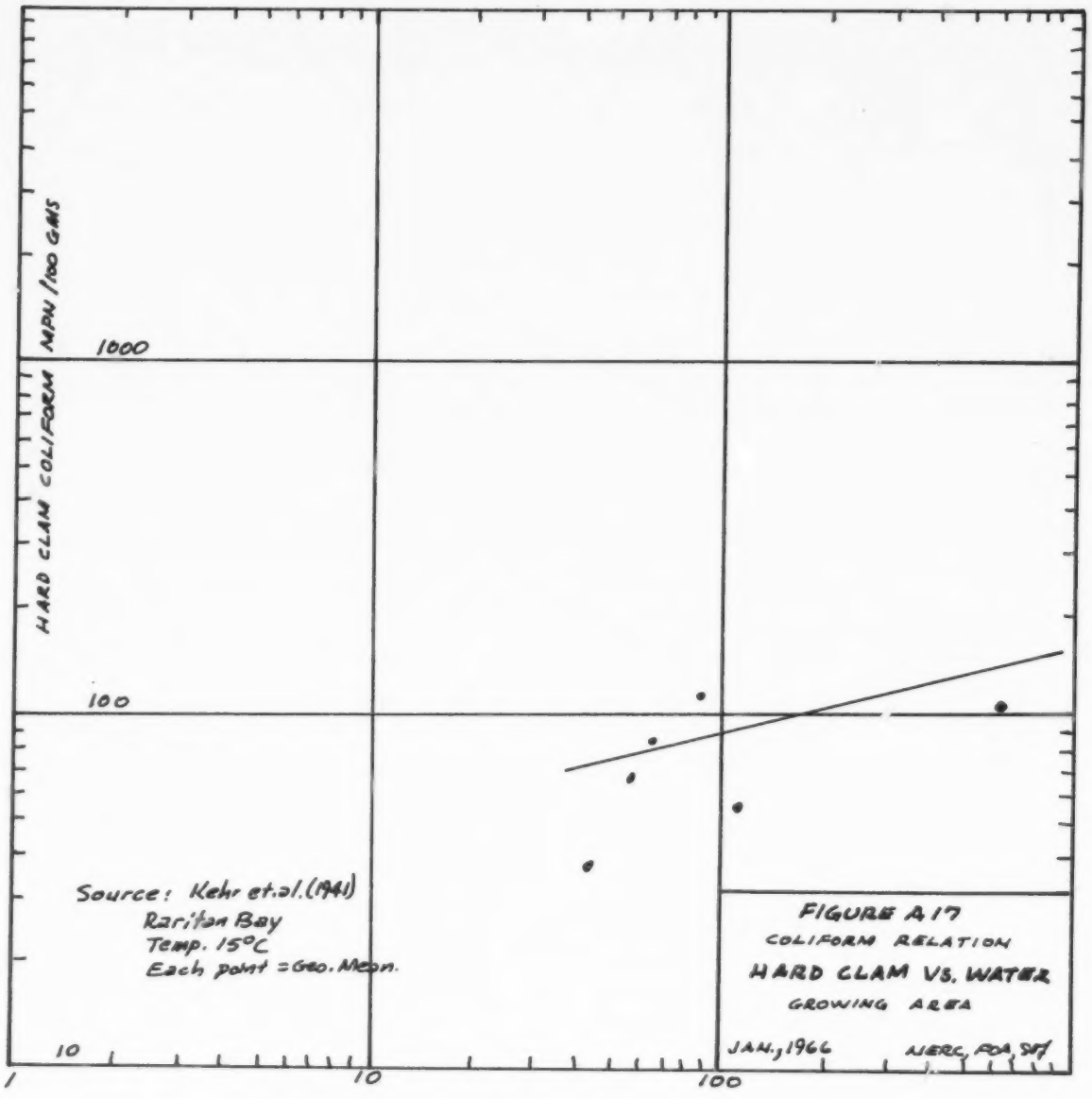
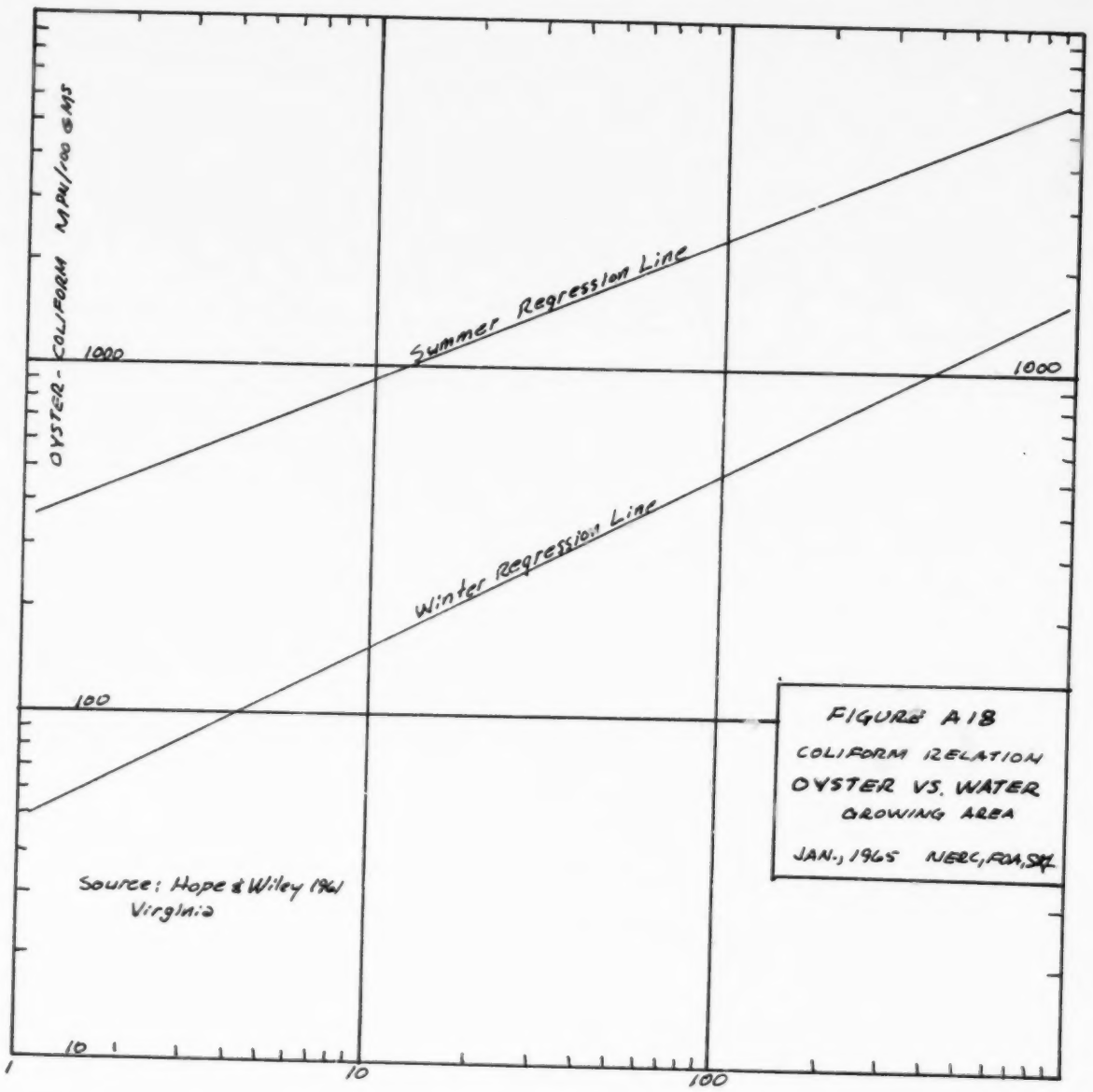


FIGURE A16
COLIFORM RELATION
SOFT CLAM VS WATER
GROWING AREA
JAN., 1965 NERC, FDA, SRT


HARD CLAM COLIFORM MPN/100 GMS
1000
100
10
1
10
100
Source: Kehr et.al.(1941)
Raritan Bay
Temp. 15°C
Each point = Geo. Mean.
FIGURE A 17
COLIFORM RELATION
HARD CLAM VS. WATER
GROWING AREA
JAN., 1966
NJERC, FOA, SPT
WATER COLIFORM MPN/100 ML


OYSTER - COLIFORM MPN/100 GMS
1000
100
10
1
10
100
1000
Summer Regression Line
Winter Regression Line
Source: Hope & Wiley 1961
Virginia
FIGURE A18
COLIFORM RELATION
OYSTER VS. WATER
GROWING AREA
JAN., 1965 NERC, FDA, SK
WATER COLIFORM MPN/100 ML

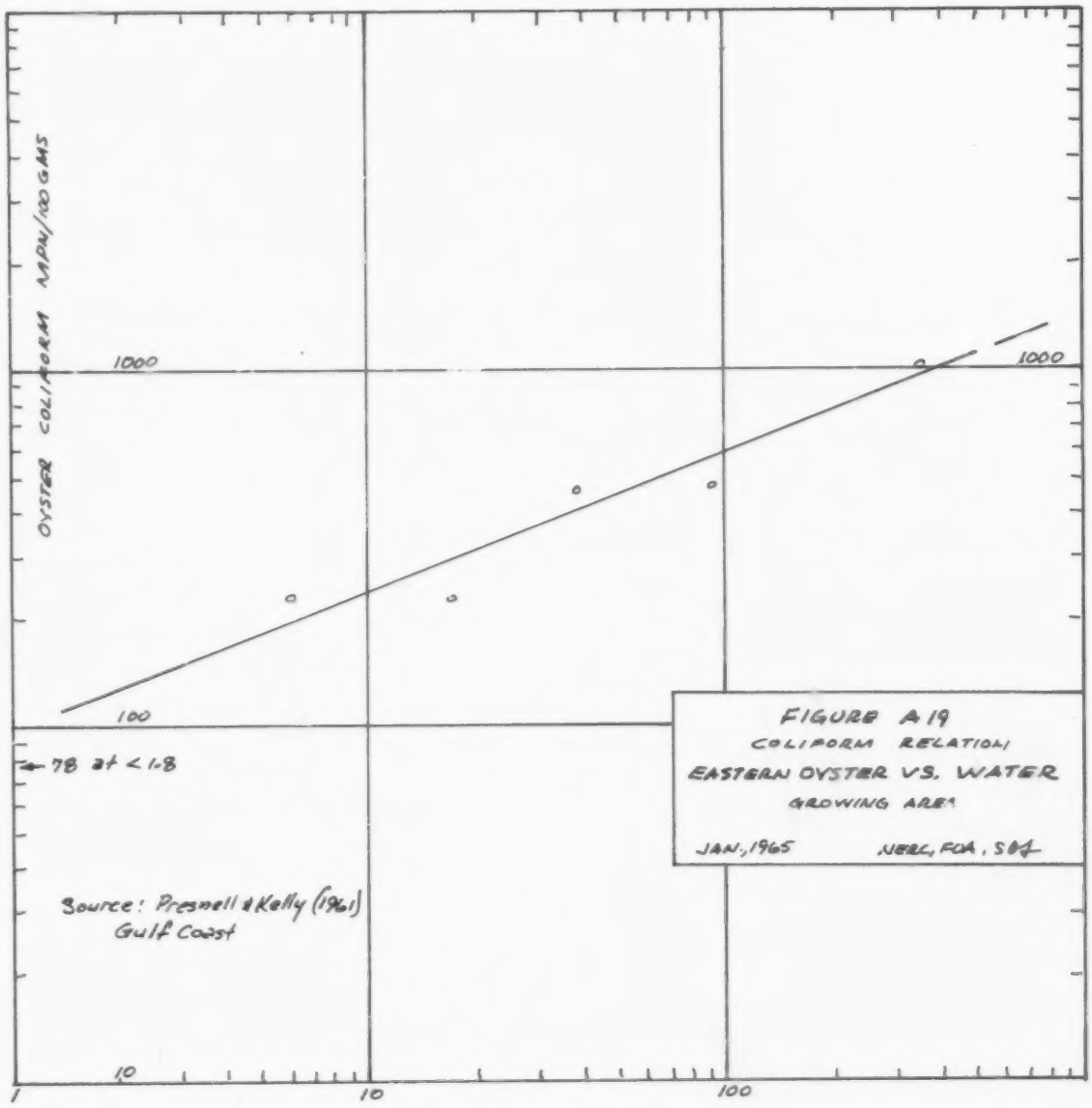
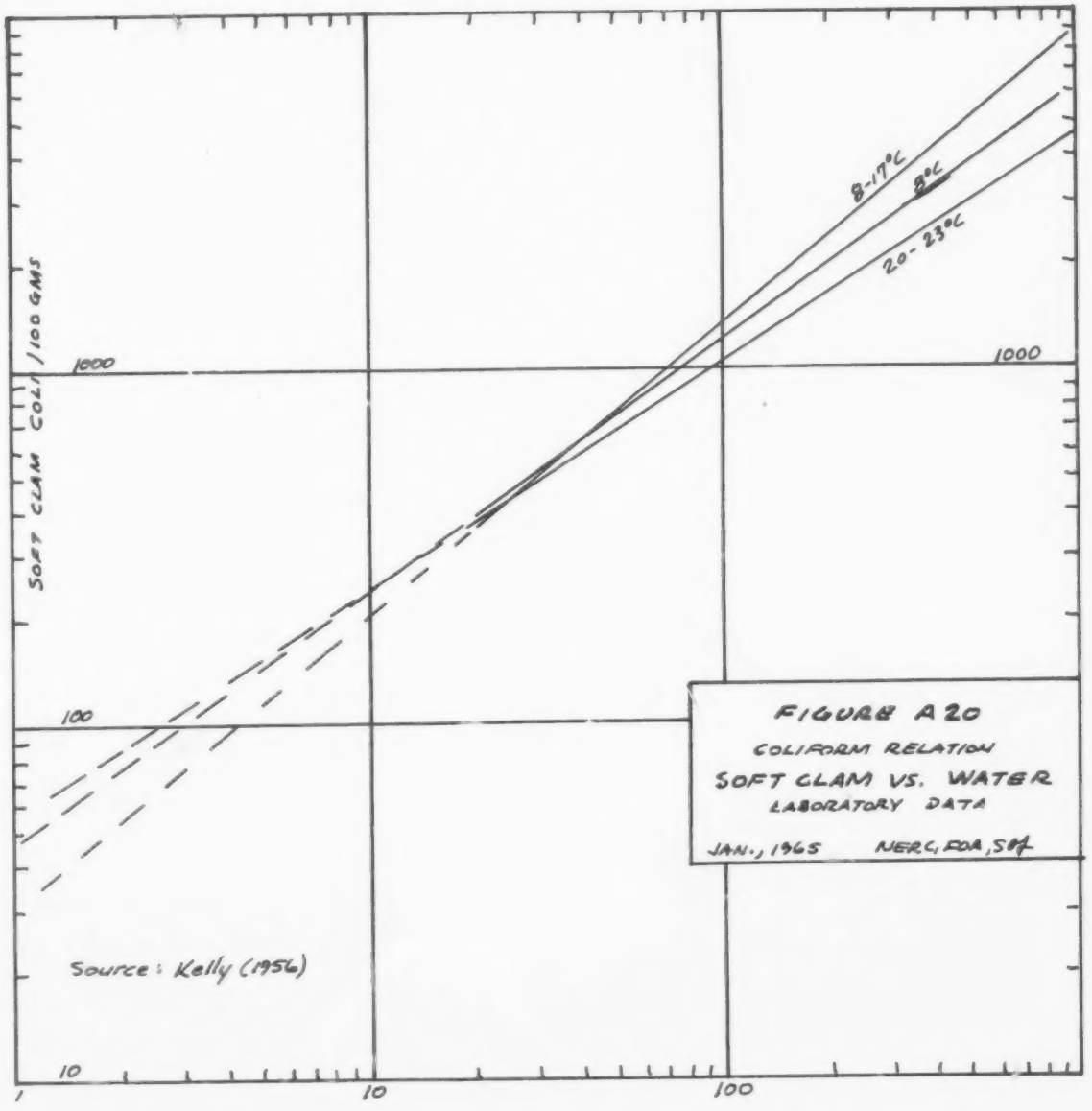


FIGURE A 19
COLIFORM RELATION
EASTERN OYSTER VS. WATER
GROWING AREA
JAN., 1965 NERC, FOA, SDA


8-17°C
8°C
20-23°C
1000
1000
100
10
1
10
100
SOFT CLAM COLI / 100 GMS
WATER COLI / 100 ML
Source: Kelly (1956)
FIGURE A 20
COLIFORM RELATION
SOFT CLAM VS. WATER
LABORATORY DATA
JAN., 1965 NERC, FDA, SA

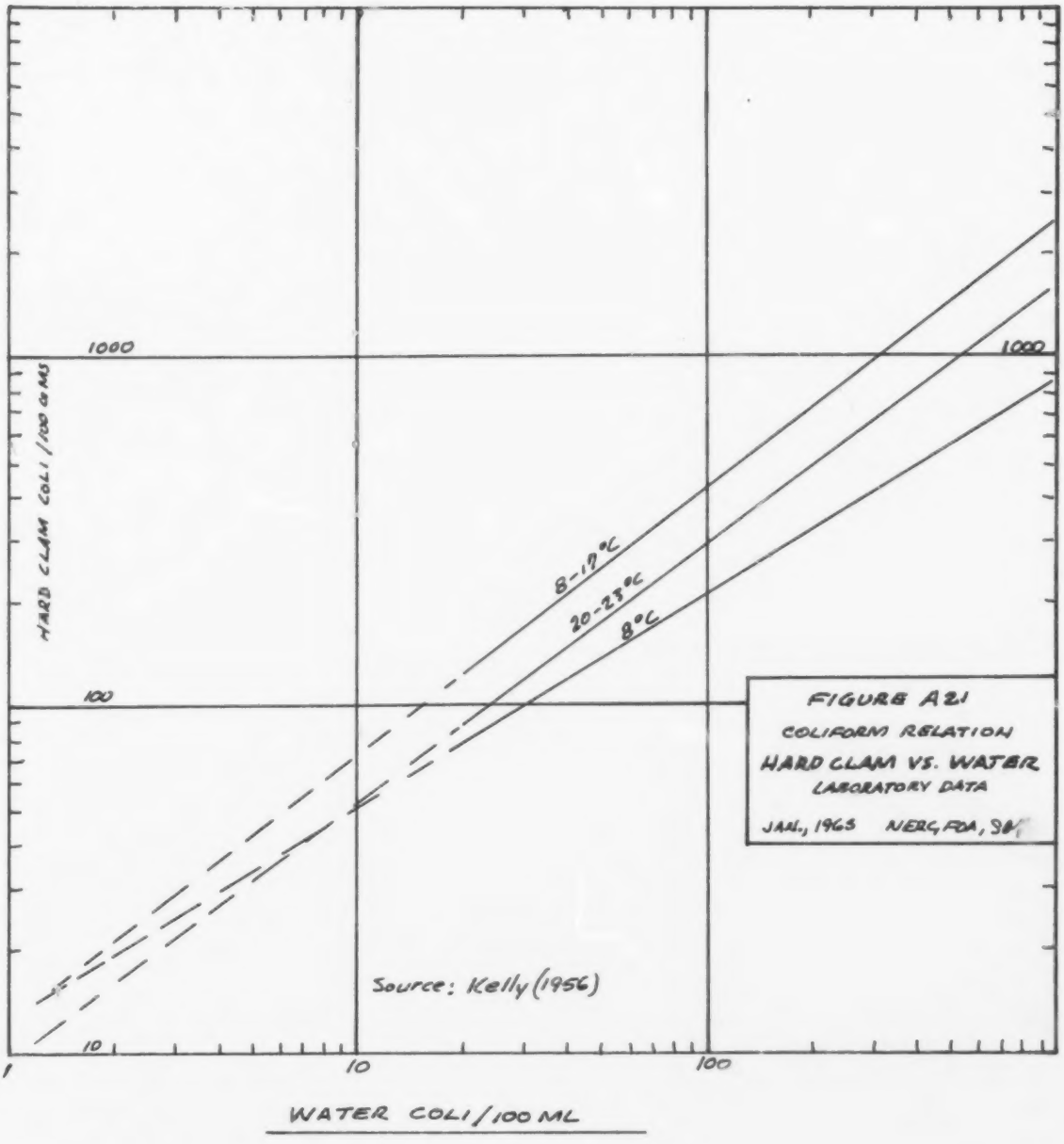
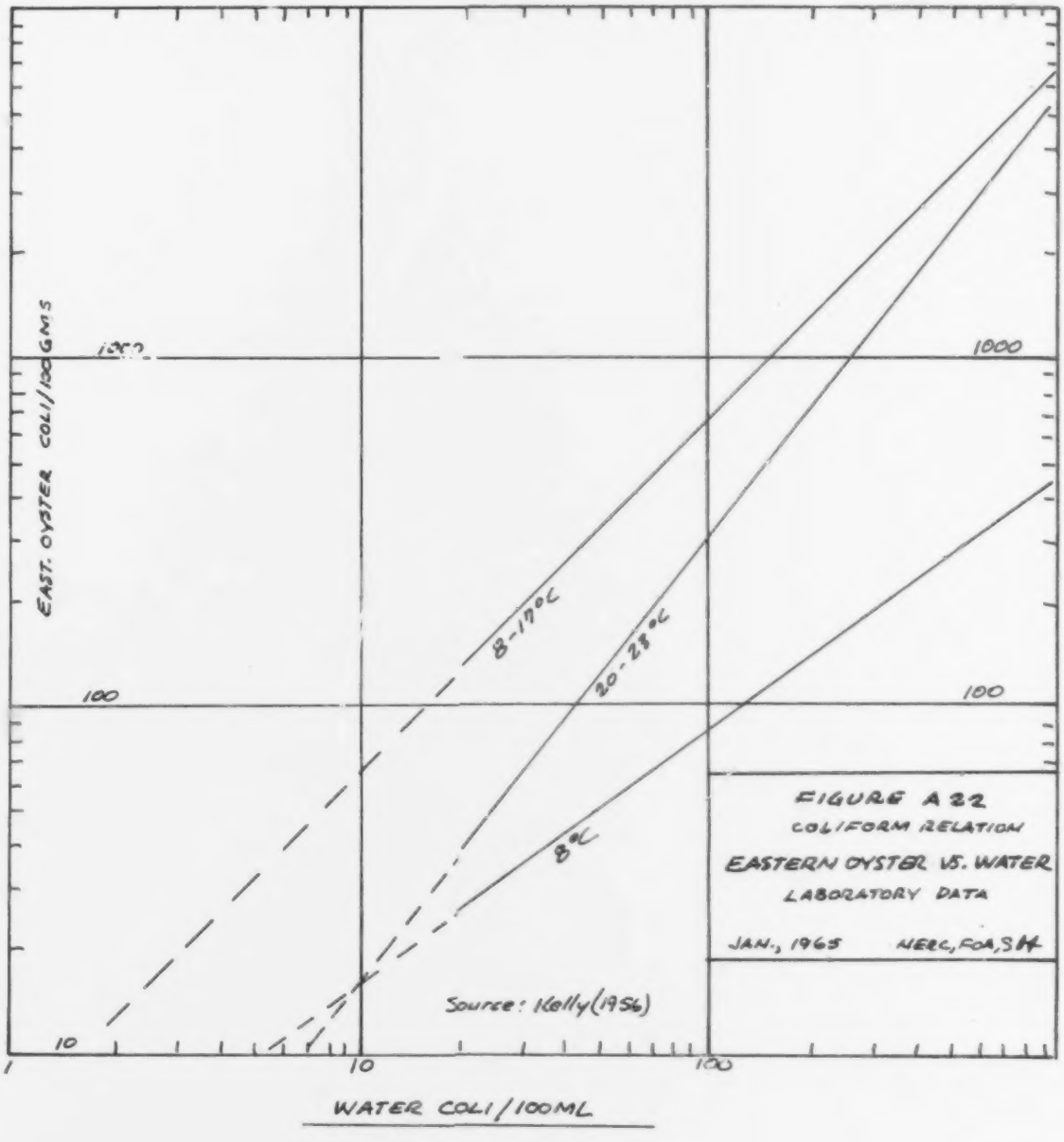


FIGURE A21
COLIFORM RELATION
HARD CLAM VS. WATER
LABORATORY DATA
JAN., 1963 NERC, FDA, 98


EAST. OYSTER COLI/100 GMS
1000
100
10
1
10
100
WATER COLI/100ML
8-17°C
20-28°C
8°C
Source: Kelly (1956)
FIGURE A 22
COLIFORM RELATION
EASTERN OYSTER VS. WATER
LABORATORY DATA
JAN., 1965 NERC, FOA, SH

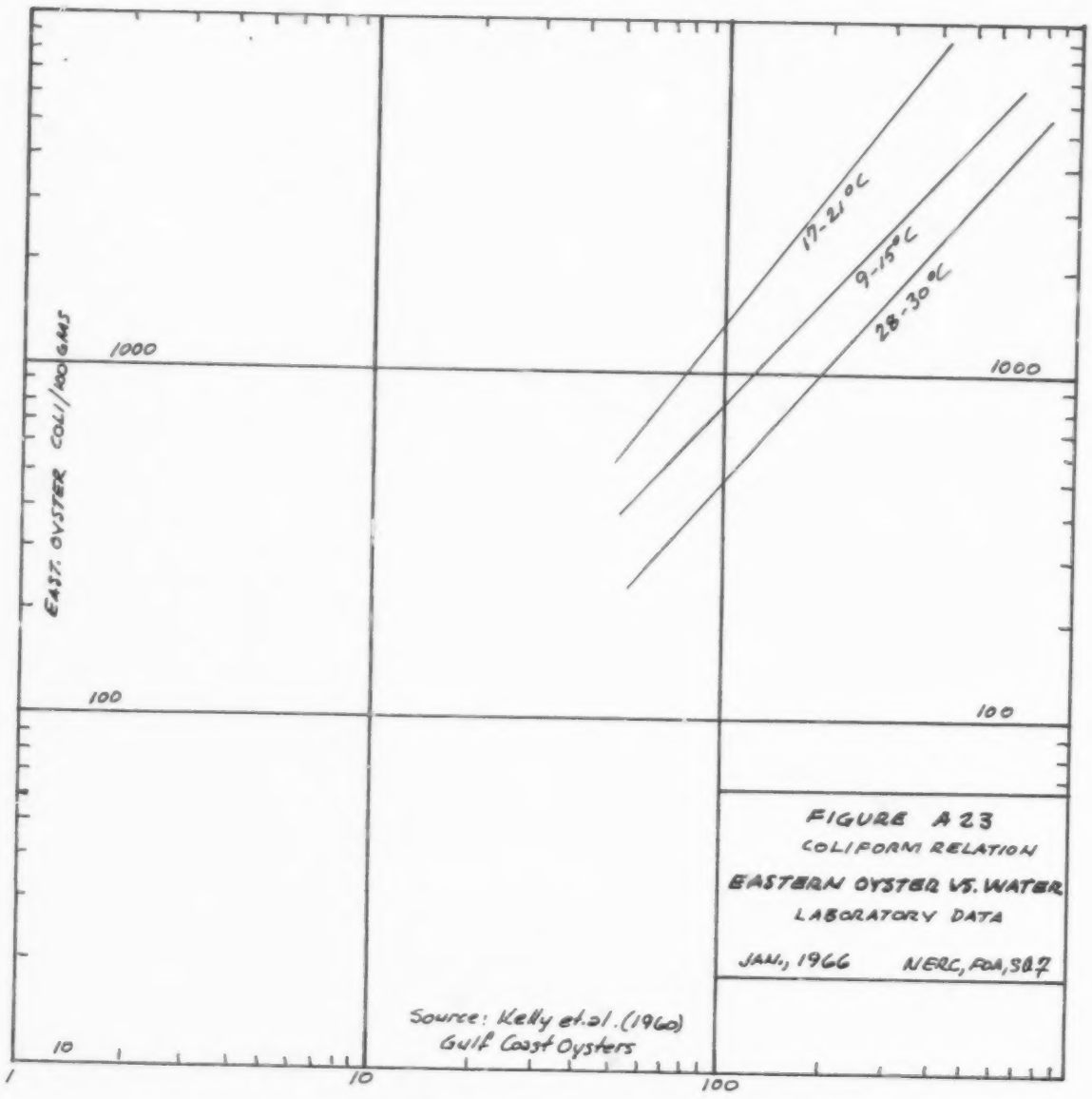


FIGURE A 23
COLIFORM RELATION
EASTERN OYSTER VS. WATER
LABORATORY DATA
JAN., 1966 NERC, FDA, SBF

Table A14. COLIFORM ORGANISMS - SHELLFISH-WATER RELATIONSHIPS

Growing Area	Surveys	Water Coli/100ml	Water E.C. /100ml	Shellfish Coli/100ml	Shellfish E.C. /100ml	Remarks
Maryland	Soft Clam	<3	---	150	<18	16 Samples
		3-3.6	---	280	<18	22 Samples - All Seasons
		9.1	---	330	20	12 Samples - All Seasons
		15-23	---	330	<18	12 Samples - All Seasons
		70	---	790	<18	16 Samples - All Seasons
Raritan Bay	Hard Clam	70	---	70	---	From the Plot - 15°C
Virginia	Oyster	<3.6	<3.6	150	18	18 Samples - Summer
		<3.6	<3.6	20	<20	13 Samples - Winter
		70	23*	2000	280*	From Regression Lines - Summer
		70	29*	420	120*	From Regression Lines - Winter
Pensacola	Oyster	<1.8	---	78	---	25 Samples - All Seasons
		2-10	---	230	---	43 Samples - All Seasons
		11-23	---	230	---	24 Samples - All Seasons
		27-49	---	460	---	22 Samples - All Seasons
		50-130	---	475	---	30 Samples - All Seasons
		>170 (Median = 350)	---	1040	---	40 Samples - All Seasons
		70	---	540	---	From the Plot
Laboratory						
Woods Hole	Soft Clam	70	---	800/900	---	Summer/Winter
	Hard Clam	70	---	250/170	---	Summer/Winter
	E. Oyster	70	---	300/65	---	Summer/Winter
Gulf Coast	E. Oyster	70	---	500/800	---	Summer/Winter

*Regression Lines for E.C. results not given in the report.

D. Derivation of Expected Levels of Attainment

1. For approved areas. The data on soft clams for growing areas and laboratory work show some consistency, in that, at the 70 coliform MPN level in water the soft clam in Maryland growing areas had a coliform MPN of 790/100 grams and for the laboratory work, MPN's of 800/100 grams for warm temperatures and 900 for cold* temperatures. The laboratory work on hard clams show for 70 MPN water, a shellfish MPN of 250 at warm temperatures and 170 at cooler temperat res, while the growing area gave a shellfish MPN of 70 at overall temperature conditions. The Eastern Oyster of Virginia waters of 70 MPN showed 2000 MPN/100 grams and 420 MPN/100 grams in summer and winter respectively. The Gulf Coast variety showed 540 for all seasons. The laboratory work on the Eastern Oyster of the northeast variety gave 300 and 65 at warm and cold temperatures respectively, and the laboratory work of the Gulf Coast gave 500 MPN/100 ml for summer and 800 MPN/100 ml for winter.

This information is summarized in Table A15 along with the proposed 24 hour percent reduction of coliform during depuration. From these data the expected levels of attainments of coliform, in depurated shellfish harvested from approved areas, are calculated.

2. For restricted areas. The level of coliform attainment for 48 hour depurated shellfish harvested from restricted areas should be approximately the same as those for 24 hour depurated shellfish harvested from approved areas.

Using the coliform remaining figures in Table A15 and the percent remaining values for 48 hour depuration given in Table A13, the general initial coliform counts in the shellfish can be derived. Table A16 presents the calculations.

---

*Refer to Table A14.

Table A15. EXPECTED LEVELS OF COLIFORM FOR DEPURATED SHELLFISH, HARVESTED FROM APPROVED AREAS

Shellfish	24 Hour Percent Coliform Remaining	Remarks	Shellfish Coliform MPN/100 ml for 70 MPN/100ml Waters	Coliform Remaining	General Expected Levels
Soft Clam	25	Maryland Grow'g Area - All Seasons	790	200	200*
		Lab. Warm Temp.	800	200	
		Lab. Cold Temp.	900	225	
Hard Clam	5	Raritan Bay Grow'g Area -- 15°C	70	3.5	
		Lab. Warm Temp.	250	12.5	<20*
		Lab. Cold Temp.	170	8.5	
E. Oyster	5	Virginia Grow'g Area - Summer	2000	100	
		Virginia Grow'g Area - Winter	420	21	
		Pensacola Grow'g Area - All Seasons	540	27	
		Lab. Woods Hole - Warm	300	15	
		Lab. Woods Hole - Cold	65	3.2	23**
		Lab. Gulf Coast - Summer	500	30	
		Lab. Gulf Coast - Winter	800	40	

* Selected Arbitrarily.
** Geometric mean of the seven listed results.

Table A16. CALCULATIONS ON LEVELS OF ATTAINMENT FOR RESTRICTED AREAS

Shellfish	48 Hour Percent Coli Remaining	Remarks	Coliform* MPN Remaining	Calculated Initial MPN	General Initial MPN
Soft Clam	10	Maryland Grow'g Area - All Seasons	200	2000	
		Lab. Warm Temp.	200	2000	2000**
		Lab. Cold Temp.	225	2250	
Hard Clam	2	Raritan Bay - 15°C	3.5	170	
		Lab. Warm Temp.	12.5	625	360***
		Lab. Cold Temp.	8.5	425	
E. Oyster	2	Virginia Grow'g Area - Summer	100	5000	
		Virginia Grow'g Area - Winter	21	1050	
		Pensacola Grow'g Area - All Seasons	2/	1350	
		Lab. Woods Hole - Warm	15	750	1100***
		Lab. Woods Hole - Cold	3.2	160	
		Lab. Gulf Coast - Summer	30	1500	
		Lab. Gulf Coast - Winter	40	2000	

* From Table A15.
** Arbitrary.
*** Geometric mean of the results.

The general expected levels of coliform bacteria in depurated shellfish harvested from restricted areas can be calculated from the foregoing; these calculations are presented in Table A17:

Table A17. EXPECTED LEVELS OF COLIFORM IN DEPURATED SHELLFISH HARVESTED FROM RESTRICTED AREAS

Shellfish	General Initial* MPN	Percent Coli Remaining	Final** MPN
Soft Clam	2000	10	200 (200)
Hard Clam	360	2	7.2 (<20)
E. Oyster	1100	2	22 (23)

* From Table A16.

** Expected values for Approved Area Shellfish given in Table A15 are in parenthesis.

Another question directly related to the problem of expected bacterial levels in depurated shellfish is: what is the coliform content of the restricted harvest area water which may give the expected shellfish levels described? The question can also be stated as: what water coliform content corresponds to the general initial coliform levels given in Table A16?

Selected ranges of the water levels are given in the following Table A18:

Table A18. COLIFORM CONTENT OF WATER IN RESTRICTED AREAS WHICH GIVES THE EXPECTED LEVELS IN DEPURATED SHELLFISH

Shellfish	General Initial MPN	Corresponding Water MPN	Remarks
Soft Clam	2000	170 - 280	Lab. Range
Hard Clam	360	80 - 230	Lab. Range
E. Oyster	1100	16 - 450	Va. Grow'g Area
E. Oyster	1100	160 - 280	Lab. Woods Hole
E. Oyster	1100	75 - 200	Lab. Gulf Coast

The average of midpoints of these ranges give a coliform value of about 200 MPN/100 ml. This is a highly qualified value, but it serves a useful purpose for describing expectations. In general, if shellfish are harvested from areas where the coliform content is greater than 200/100 ml, the final levels of coliform in shellfish depurated for 48 hours will not consistently approach those derived. It is noted that there is a wide variance for the Gulf Coast variety of the eastern oyster. For a general initial coliform level of 1100/100 ml in the oyster, the water coliform for all seasons will be about 460/100* ml, more than twice as much as the 200/100 ml.

Because of the limited data involved in this discussion it can be expected that wide variations in similar data will occur for different locales, climates, sources of waste, etc. But the foregoing analysis is the type required to establish prediction systems and maintain strict control over operation of depuration plants.

Interim guidelines with a large safety factor can be derived from the information available. An interim guideline may be stated as follows: Unless studies are conducted to show in what depuration time consistent low acceptable bacterial levels of coliform will occur, then the water in restricted areas, from which shellfish are to be depurated for not more than 48 hours, should not exceed 200 coliform MPN/100 ml.

---

*Figure A19.

III. Establishment of Some Plant Design Factors

A. Sea Water Flow

Sea water flow was discussed in the section on oxygen, Appendix I. The flow rate derived was 1 gallon per minute per bushel.

B. Tank Loading Information

1. At Biddeford Pool, Maine, (Goggins, et al, 1965) experiments with soft clams; 12 bushels were depurated in a tank of size 4 ft x 8 ft x 2 ft deep (480 gallons), about 40 gallons or 5.4 cubic feet per bushel.

2. At Bristol, Rhode Island, (Canario & Tubman, 1965) experiments with hard clams; loading of clams in the range of 28 to 112 gallons per bushel or 3.8 to 15 cubic feet per bushel had no measurable affect on depuration.

3. Wood (1961) recommends about 125 gallons (16.9 cu.ft.) of tank capacity for about every 500 oysters (O. edulis) or about 2 bushels. He also recommends a loading of 50 oysters per square foot of basket (a layer of about 3-4 inches).

4. Conclusions. Based on the above the following factors are recommended to insure adequate flow through and around baskets containing shellfish and allows for hosing down operations and also sufficient freeboard.

Table A19. TANK SIZE AND LOADING FACTORS

Shellfish	Gross Tank Size	
	Gallons/bushel	Cu.Ft./Bushel
Soft Clams	40	5
Hard Clams and Oysters	60	8

IV. Shellfish Depuration and Viruses

The question as to the effectiveness and reliability of the depuration process for the removal of viruses has emerged since it has become apparent that pollution of shellfish beds has increased and that shellfish harbor virus.

It has been shown by various authors[1] since the turn of the century that the typhoid bacilli, coliform organisms, and other bacteria are removed to a large extent from shellfish when they are placed in a clean environment. Times for complete removal have varied from several hours to several weeks. The fact remains, that, if shellfish are feeding naturally and in general acting normally during a depuration process the contents of the gut are removed. The recent depuration experiments, the results of which are given in Figures A7 thru A15, show that shellfish do release coliform and Salmonella. For hard clams and oysters, some results show no detectable coliforms after depuration despite high initial levels.

Levels of virus in shellfish are reduced by depuration as is shown below. However, the public health significance of the remaining level (whether low or undectable) is at present a moot point. The number of virus particles remaining in a shellfish can be tested to only certain levels by present techniques. Whether or not these numbers, if ingested by an individual is an infectious dose has been difficult to ascertain.

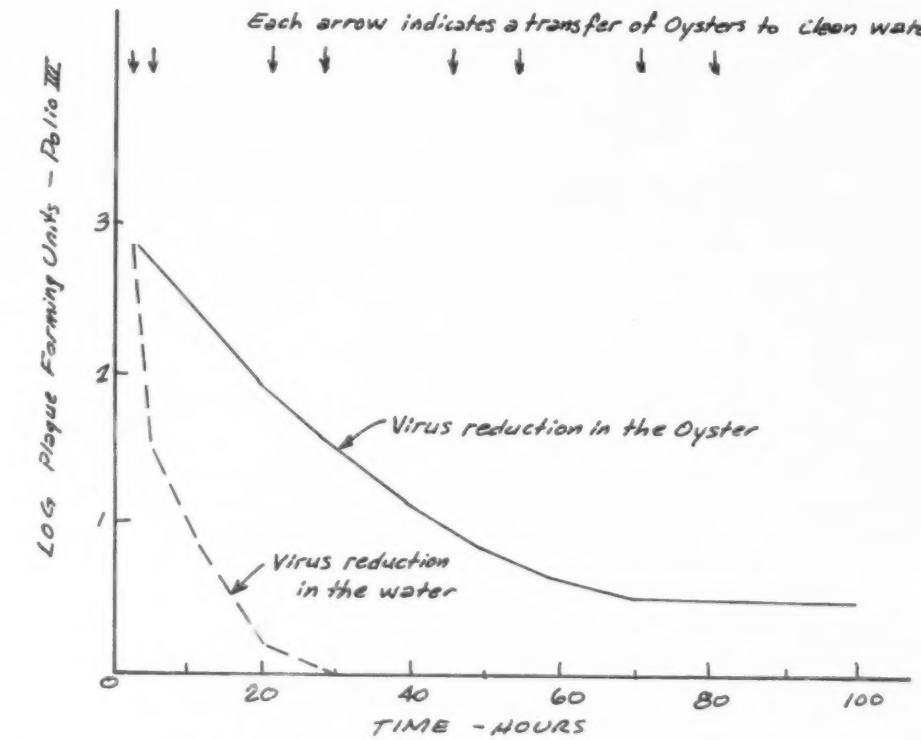
Several significant works on depuration of viruses by shellfish have been reported. They are described in chronological order in the following paragraphs.

Crovari (1956) observed the depuration of Polio II from the mussel (Mytilus edulis). The virus was found at 24 hours, but not at 48 hours. No levels were reported; only the presence or absence.

Hedstrom and Lycke (1963) reporting on oysters (probably O. edulis) presented a chart showing rate of removal of Polio III virus. The curve is presented in Figure A24. This curve describes the levels of virus after certain transfers of the oysters to a clean water after the denoted period. This information showed depuration of Polio III, to some level, to take place even under conditions which were not conducive to a normal level of physiological activity.

---

[1] Appendix V



After: Hedström & Lycke (1965)

FIGURE A 24

DEDURATION OF VIRUS REDUCTION OF POLIO III

JAN., 1965 NERC, FOA, Sat

Atwood, Cherry and Klein (1964) experimenting with the hard clam, showed the uptake, retention and depuration of Coxsackie B-5. The curve is depicted in Figure A25.

For the interpretation of the results of any experiment on depuration, there are several considerations which must be taken into account in addition to the environmental factors.

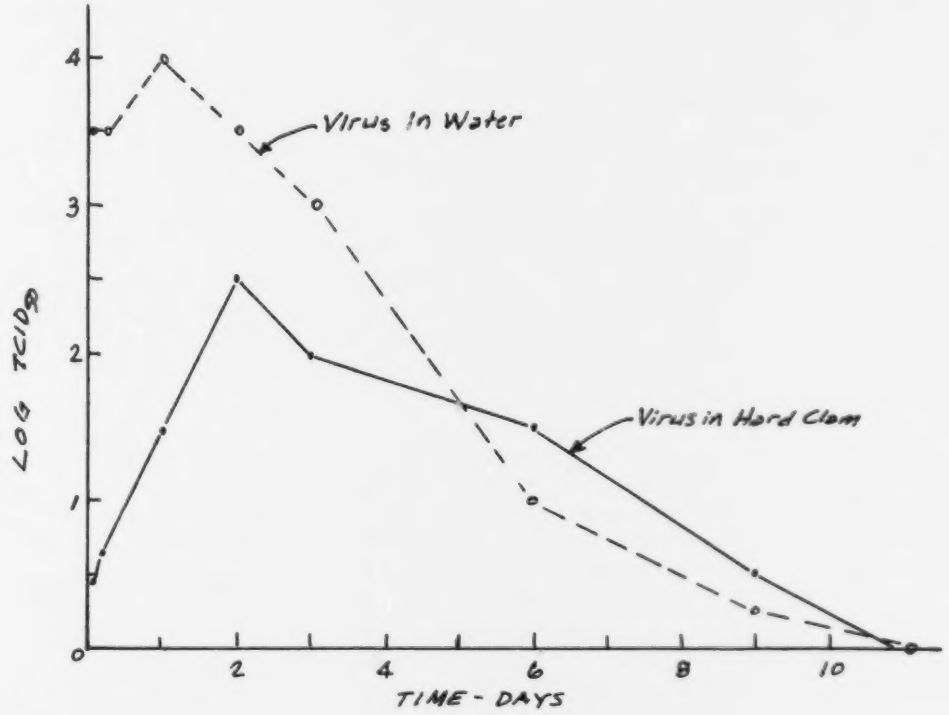
The doses of virus that are given to the shellfish are of concern. To demonstrate viruses in shellfish, large doses are required; usually much larger than would occur in nature. Large experimental water doses may be necessary to demonstrate a well-defined reduction curve; but in waters polluted at the restricted area level ($\leq$700 coliforms/100 ml) viruses are difficult to find by present techniques.

Furthermore, as has been reported by Stauber (1961) not only are portal and mode of entry of a parasite important in its effect of immunity response, but also the size of the infecting dose. It is logical to assume that a large dose of virus will require a longer time for complete elimination than a smaller dose. More virus particles are available for adsorption to the mucus with a large dose than with a small dose. A small dose in nature may be a single infectious unit (to man) of a virus which occasionally contacts a shellfish during its stay in the harvest area.

Metcalf and Stiles (1965) showed that within practical detecting limits, there is a threshold concentration of Coxsackie B-3 and Polio I, in the environment, below which virus could not be detected in eastern oysters. Below a log $TCID_{50}$* of 4.6 in six liters of sea water, e.g., Coxsackie was not detected in the soft parts of oysters. Other observations included: a) Coxsackie B-4 virus was isolated from oysters harvested when the sea water coliform MPN was a maximum of 2850/100 ml; no viruses were isolated when the maximum MPN was 185/100 ml; b) there was no detectable multiplication in oysters of Coxsackie B-3 and Polio I; c) viruses were detected after 28 days in oysters dry-stored at 5°C.

Mitchell, et al, (1965) studying uptake and depuration of Gulf Coast oysters (C. virginica) found rapid uptake of Polio I virus to a concentration equivalent to the water, and a self-

*$TCID_{50}$ is an expression of a virus titer. In general, it is a calculated value, derived from a tissue culture tube technique.



After: Atwood, Cherry and Klein (1964)

FIGURE A25

FATE OF COXSACKIE B5 VIRUS IN HARD CLAMS

JAN., 1965 NERC, FOA, SIM

cleansing to levels undetectable by the method in 48 hours in one experiment. In another experiment 2 PFU/gm persisted for 96 hours after zero-hour. Over 95% of the virus was eliminated within 8 hours, however. Virus results paralleled coliform results.

Liu, et al, (1965) using Polio I virus and hard clams as a working model for depuration studies, showed rapid uptake and then elimination rates paralleling coliform. Viruses were detected in the shellfish after 48 hours with a high pollution level, but viruses were undetected at 24 hours with a low pollution level in one given experiment.

Both Liu and Mitchell used open sea water systems with ultraviolet light treated water. An open system eliminates the shellfish's metabolic waste factor that is present in experiments conducted in closed or static systems.

Liu further found: a) increasing amounts of virus in fecal matter with time; b) virus particles were not chemically bound to cells nor were they intracellular. Both these findings indicated that virus in the digestive system was removed readily.

Depuration of viruses is partly confirmed by experience in Europe where there has been no epidemiological evidence of virus diseases transmitted by shellfish which have been depurated and originally came from polluted areas.*

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*See "Proceedings - 5th National Shellfish Sanitation Workshop," November 17-19, 1964. "Depuration -- The National Posture" by E. T. Jensen, 71-74.

V. Synoptic History of Depuration and Depuration Research

The following is a partial chronological account of research which can be associated with depuration. Brief remarks and statements on findings are given. It is noted that there is a period of about nine or ten years between Galtsoff (1946) and Kelly (1961) during which only scattered work was done. It appears that Galtsoff's work showing that chlorination inhibited shellfish activity dampened efforts on depuration. However, the idea of using UV treatment of sea water occurred at a time when it appeared that effort on depuration was seriously needed.

Date	Author(s)	Shellfish	Remarks
1899	Herdman & Boyce (Great Britain	Oysters	B. Typhosus diminished or disappeared from lab. infected oysters 1 - 7 days.
1905	Klein (Great Britain)	Oysters, Mussels	Found rapid diminution of B. Typhosus.
1908	Johnstone (Great Britain)	Mussels	May be the first relaying; also verified Klein's work.
1910-1913	Fabre-Domerque (France)	Oysters	Artificial sand-filtered sea water: B. coli removed in 3-6 days; B. Typhosus removed in 3 days.
1913	Bodin and Chevrel (France)	Oysters	Confirmed Fabre-Domerque's work: 6 days for complete purification.
1915	Dodgeson (Wales)	Mussels	Started Work.
1916	Wells (USA) (USPHS)	Oysters	Used Ca OCl_2 - 0.25 - 0.63 ppm Cl_2: for treatment: 4-29 hours good results with B. coli reduction.
1921	Carmella (USA) (USPHS)	Oysters	With hypochlorite treatments 90% removal of B. coli in 18-24 hours: Concluded that treatment (depuration) provides additional safety factor.
1923	Wells (USA)	Oysters	Disinfection by electrolysis at Linwood, L.I., New York. 2 concrete tanks - 400 bush/day successful disinfection.
1926	Krumweide	Oysters	10 ppm Chlorine in sea water: 99% removal of B. Typhosa after 6 treatments. Small numbers persist for long periods.
1927	Massachusetts Health Dept.	Soft Clam	Experiments at Newburyport with chlorine treatment.

Date	Author(s)	Shellfish	Remarks
1928	Krumweide	Oysters	3 weeks for 100% removal of typhoid.
1928	Dodgeson (Wales)	Mussels	Published the classical "Report on Mussel Purification."
1929	Violle (France)	Oysters	Used Ozone to sterilize water: found 5-6 days to sterilize oysters. Used B. typhosus, B. coli, B. proteus, V. cholerae, B. pyocyaneus, B. dysenteriae. 15 days at most.
1929	Wells (USA)	Oysters	Treatment of oysters in chlorinated sea water has been shown experimentally and in a commercial plant to be a safeguard.
1930	- - - - - -	Soft Clam	Construction of Newburyport, Mass., plant for purification of soft clams.
1934	Nelson, J. R. (USA)	Oyster	Closed system all right for conditioning of good product; opposed to use for depuration of a doubtful product.
1937	Measer & Reese (Virginia)	Oyster	Fill and draw method for oyster conditioning at cold temperatures.
1938	Petrilli (Italy)	Mussels	Bacillus typhosa not demonstrable in mussels after 48 hours. Phagocytosis not a significant mechanism in self-purification.
1938	(Shuster, 1952)	Soft Clam	Desanding in a New Jersey Plant.
1946	Galtsoff (USA)	Oysters	Chlorination affects pumping and activity in concentrations as low as 0.01 ppm.
1955	Arcisz and Kelly (USA)	Soft Clam	Soft Clams depurate over a wide range of temperatures in water with low coliform counts.

Date	Author(s)	Shellfish	Remarks
1956	Crovari (Italy)	Mussel	Polio I virus not present after 48 hours of laboratory depuration.
1961	Kelly	----	Disinfection of sea water by ultraviolet light.
1961(a)	Wood (England)	Oysters	Experiments and design for depuration plants using ultraviolet light.
1961	Kelly	Oysters	Demonstrated rates of decrease of Coli of Pacific Oysters (at Purdy, Washington).
1963	USA - R. I.	Hard Clam	Bristol, R.I., experiment with hard clam.
1963	Hedstrom & Lycke (Sweden)	Oyster	Virus takes several days to be removed in a static-type system.
1963	Bito and Takase (Japan)	Oyster	Self-decontamination shown with radio-active labeled Coli.
1963	Maine, Sea & Shore Fisheries	Soft Clam	ARA-sponsored studies at Biddeford Pool and Boothbay Harbor, Maine.
1964	Maine	Soft Clam	Construction of a private Depuration Plant and Certification by State.
1965	Metcalf and Stiles, Mitchell, et al, Liu, et al	Various	Work with virus, accelerated by universities and USPHS and continuing.
1966	Beck, et al	Various	Northwest Shellfish Sanitation Research Planning Conference.

VI. Economics, Financing and Management of Depuration Plants

A. General Costs

Tables and charts showing relative fixed costs and operating costs for depuration plants have been prepared so that administrators, engineers and scientists* may determine ranges applicable to their needs. These cost figures can also be used to estimate needs for pilot plant studies. These costs are applicable as of 1964 and should be adjusted accordingly for later years by the appropriate Construction Cost Index**

Costs of concern are broken down into Initial Costs, Operating Costs, and Break-even Costs; Figures A26 to A29 have been prepared from the information given in Tables A20 to A24.

Some of the designing factors given in Table 6 were used to calculate these costs: specifically; the tank costs were derived from surface area of tanks; Purdy units and the Maine wash & cull machines have cost $150 and $800 respectively; piping requirements will differ for each location but certain unit amounts were estimated; cost of building assumed a warehouse-type construction; cost of baskets and cold storage units of course vary with type, material and construction and number, but conservative estimates are given.

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*In government and industry.

**The usual index is the Engineering News Record Construction Cost Index (ENR); 1913 = 100, 1964 = 950, July 1965 = 980.

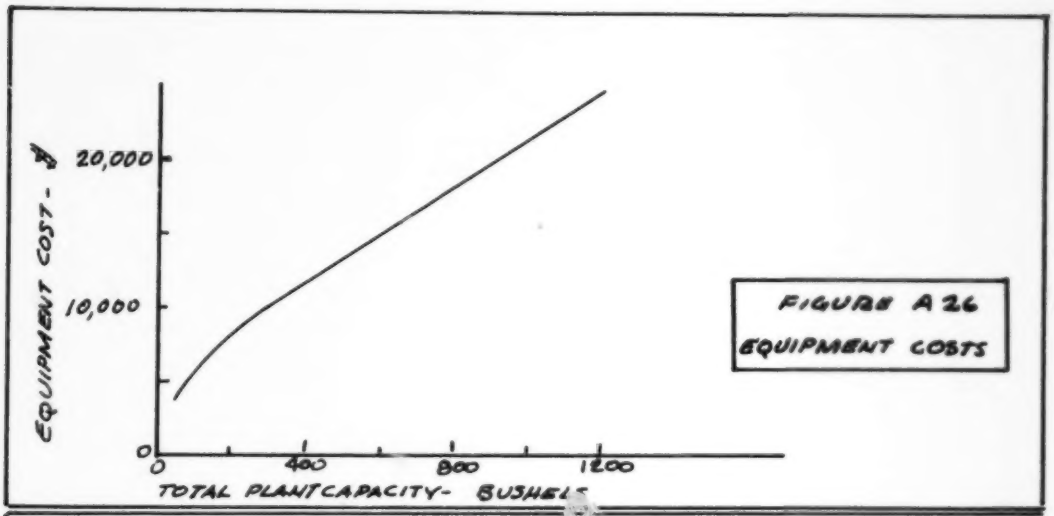

FIGURE A 26
EQUIPMENT COSTS

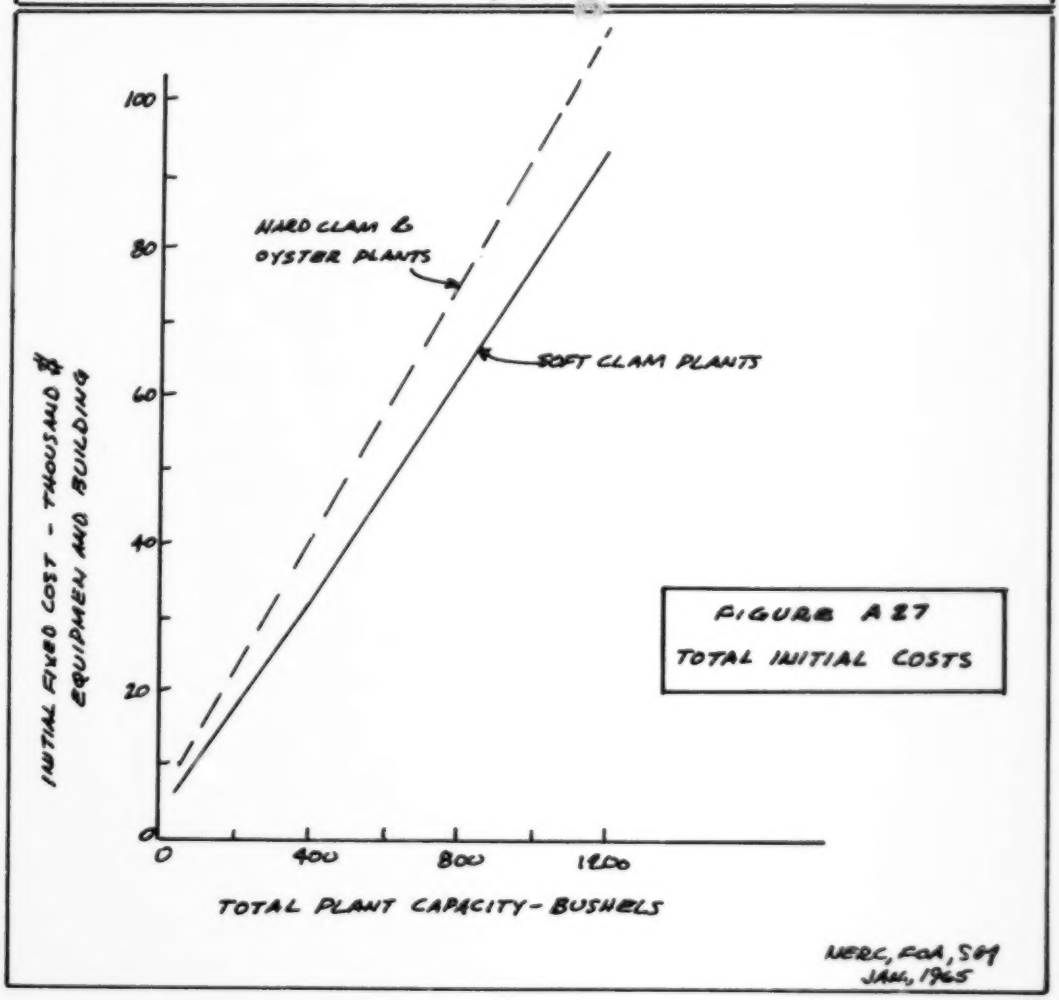

FIGURE A 27
TOTAL INITIAL COSTS

NERC, FOA, SG
JAN, 1965

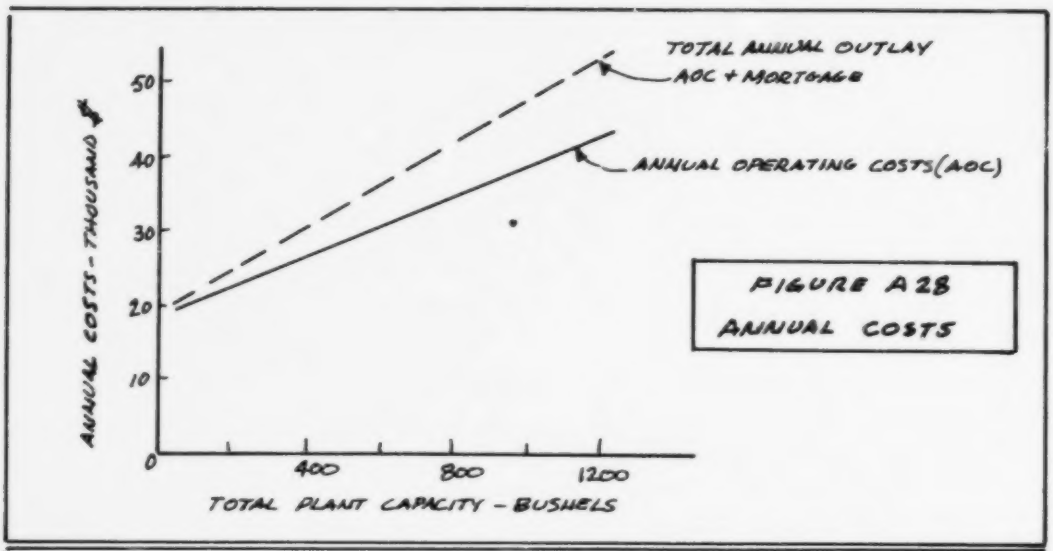

FIGURE A28
ANNUAL COSTS

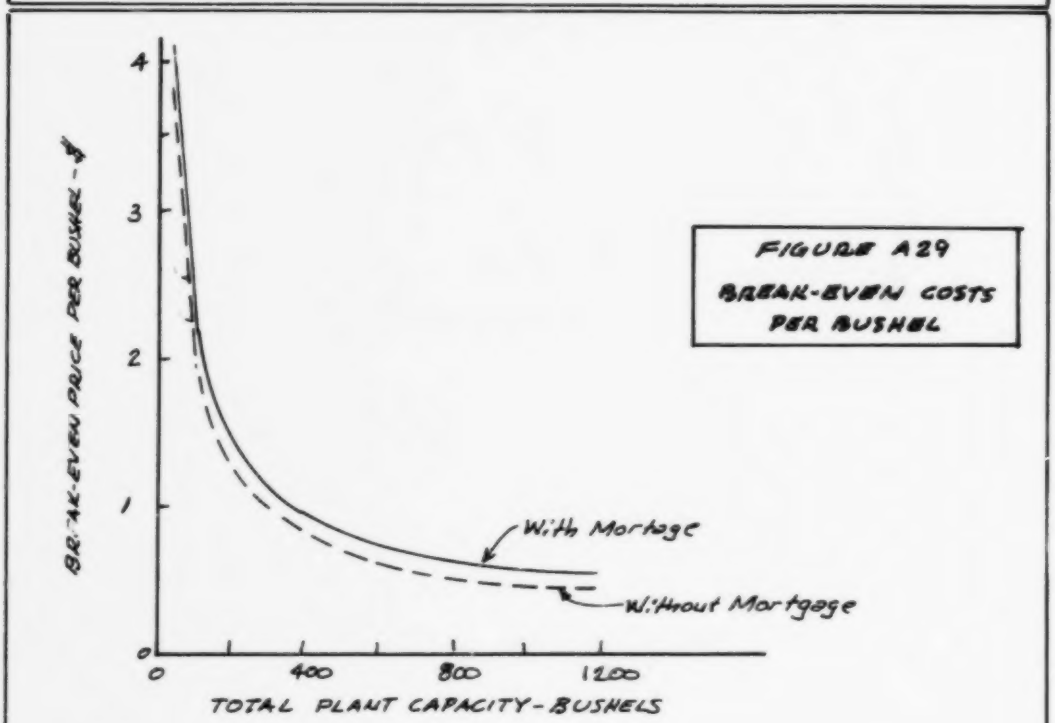

FIGURE A29
BREAK-EVEN COSTS
PER BUSHEL

NERC, FOA, SOT
JAN., 1965

Table A20. INITIAL COSTS

EQUIPMENT
FOR SOFT & HARD CLAMS and OYSTERS

Total Plant Capacity Bushels	Tanks* $	UV Units $	Storage $	Wash & Cull Machines $	Pumps, Piping, Intakes $	Baskets $	Total Equipment
50	200	300	900	800	1,200	600	4,000
100	400	450	1,000	800	1,400	1,200	5,250
200	700	900	1,200	1,600	1,700	2,500	8,600
400	1,200	1,800	1,300	1,600	2,200	3,000	11,100
800	3,000	3,500	1,600	1,600	3,300	6,000	19,000
1,200	4,000	5,300	2,000	1,600	4,300	7,500	24,700

*Tanks for soft clams cost about 20% less but the difference is not significant in the total cost of equipment.

Table A21. TOTAL INITIAL COSTS - BUILDING AND EQUIPMENT

Total Plant Capacity Bushels	BUILDING COST		TOTAL INITIAL COST	
	Soft Clams	Hard Clams & Oysters	Soft Clams	Hard Clams & Oysters
50	3,200	4,400	7,200	8,400
100	5,500	7,700	10,750	12,950
200	10,000	14,900	18,600	23,500
400	20,500	29,500	31,600	40,600
800	38,100	57,300	57,100	76,300
1,200	70,600	85,300	95,300	110,000

Table A22. ANNUAL OPERATING COSTS (AOC)

Total Plant Capacity Bushels	Labor Cost $	UV Units Maintenance	Maintenance and Heating 10% of Building*	Total Annual Operating Cost $
50	19,000	400	440	19,840
100	19,000	600	770	20,370
200	19,000	1,100	1,490	21,590
400	24,000	2,200	2,950	29,150
800	24,000	4,200	5,730	33,930
1,200	29,000	6,400	8,530	43,930

*Use Building Cost for Oyster.

Table A23. MORTGAGE CONSIDERATIONS

Total Plant Capacity Bushels	Initial Cost Quahog Plant	20 yr. Mortg. Principal & Interest	Cost* per Year	Total Annual Outlay --- Mortg. & AOC
50	8,400	16,800	840	20,680
100	12,950	25,900	1,300	21,670
200	23,500	47,000	2,350	23,940
400	40,600	81,200	4,100	33,250
800	76,300	152,600	7,630	41,560
1,200	110,000	220,000	11,000	54,930

*These figures represent an amortization at almost 7.5%.

Table A24. PRICE DIFFERENCE PER BUSHEL -- BUYING -- SELLING TO BREAK EVEN -- RUNNING AT CAPACITY

Total Plant Capacity Bushels	(1) Expected Daily Output Min. Bushels	(2) Expected Annual Output Bushels	Annual Cost		Break-Even Cost Per Bushel	
			Without Mortgage $	With Mortgage $	Without Mortgage $	With Mortgage $
50	20	5,000	19,840	20,680	3.98	4.15
100	35	8,800	20,370	21,670	2.32	2.46
200	70	17,500	21,590	23,940	1.23	1.37
400	130	32,500	29,150	33,250	0.90	1.02
800	270	67,000	33,930	41,560	0.51	0.62
1,200	400	100,000	43,930	54,930	0.44	0.55

(1) See Table 6.

(2) Assumes 250 days/year.

B. Management of Depuration Plants

The following describes in a general manner the management practices of depuration plants in certain locales. The European information was derived from notes by Messrs. Arnold Tubman and C. B. Kelly, USPHS, who visited these facilities in 1962.

Place	Technical Control and Operation	Ownership	Assistance
Conway, Wales	County	Municipal	Federal-Ministry of Fisheries
Other English	Private	Private	Federal or County
Saint Mandrier, France	Cooperative-Industry-Private		Federal
Sete, France	Cooperative-Industry-Private		Federal
Tarragona, Spain	Spanish Ministry of Health		
Rosario, Portugal	Federally Built		
Phippsburg, Maine	Private-State	Private	State-Small Bus. Loan
Newburyport, Massachusetts	State	State	Fees collected from harvesters and communities where soft clams are harvested.

VII. Shellfish Quality Standards In Depuration Plants

A. Introduction

No standards per se have been set in this report, but the following paragraphs describe how standards may be arrived at.

In any quality production process there are three considerations to be dealt with in setting quality standards, tolerances and/or specifications. They are the testing methods, the capability of the process, and the use of the product. In shellfish depuration plants, the testing methods have been standardized as the coliform and E.C. gas positive MPN tests.

The basic MPN tests have shown bacterial reductions in the order of one to several log cycles depending on species and other conditions. The use of the product, human consumption, means that stringent standards are called for. Quality standards for depuration, therefore, fall into the classical pattern of -- lots that do not meet standards should be rejected otherwise a health hazard has been released: and lots that meet standards should, of course, be accepted, otherwise there is a waste of money.

The purpose here is to discuss the several facets included in quality control for depuration plants.

B. Description of Expectations

1. For shellfish harvested from approved areas the minimum time for depuration is 24 hours and the MPN test expected levels are: Soft Clam, coliform MPN/100ml of 200, for the hard clam <20, and oyster, 23.

2. For shellfish harvested from restricted ($\leq$700/100ml) areas the minimum depuration time should be 48 hours and the bacterial levels after depuration should be about the same as the above.

3. Shellfish, harvested for processing in depuration plants when the mean coliform MPN of the water exceeds 200/100ml but $\leq$700, and if pilot tests are not conducted, should be depurated for 72 hours.

4. Shellfish should not be harvested from prohibited areas for tank depuration as described in this report.

Of note in the above is the fact that an imposition is made on the quality of the incoming shellfish indirectly by means of specifying the quality of the growing area water. The incoming quality must be quantified and standardized as well as the outgoing because of the unknowns associated with harvesting from highly polluted waters; these unknowns being viruses and toxic chemicals.

C. Meeting of Standards

In addition to the reason that the incoming quality may be poor, there are several other reasons why depuration standards may not be met. They are:

1. The shellfish were not actively feeding, pumping, etc.;

2. Recontamination of tanks because of faulty procedures or breakdown in bacterial treatment units;

3. A bad sample: e.g., a dead shellfish with a high MPN homogenized with several acceptable shellfish may reflect on the final result;

4. The MPN test itself has a high degree of variability (therefore enough samples should be taken to offset this);

5. Initial standards may be based on limited data and quality standards are set to high.

After a backlog of information has been accumulated and analyzed a refining of standards, if warranted, should take place. An effort to meet the proposed levels should be made because plants may release shellfish to market before final bacterial results are in. The calling back of lots to be re-depurated would be costly if not impossible in certain situations.

D. Data Handling in Depuration Plants

The main purpose of testing is to determine if process standards are being met. Testing will determine if the depuration plant is being run properly, if the sea water is of good quality, etc.

There are two interpretations which can be made on bacterial data. The first is a comparison of day-to-day results to determine if a real reduction of coliform organisms took place in a given lot. The second is a day-to-day comparison of final outgoing quality to see if there are any deteriorating trends because of a) incoming quality, b) the method of testing, c) poor operational and handling procedures and d) too many indicated bad lots being accepted.

E. True Bacterial Depuration

True bacterial depuration is depicted in Figure A30, which shows hypothetical reductions of the mean coliform MPN for several runs for the initial or zero-hour results, the 24-hour results and the 48-hour results. Note that the slope of the lines decreases with increasing time. This should occur if the depuration sea water is of a uniform low bacterial content and if there is no recontamination of tanks. With increasing time, the slope should approach that of the theoretical distribution for the MPN test itself (which depends on the number of tubes).

Probability plots of accumulated data should be made frequently. The guideline for determining how often to plot is the number of samples that are being taken. Each line should be represented by at least 15 points and preferably 20 to 25. Therefore, if a particular plant makes 5 zero-hour MPN determinations in a day there would be sufficient information to plot a line in about a week.

It is therefore recommended that plots such as those in Figure A30 be made on a weekly basis.

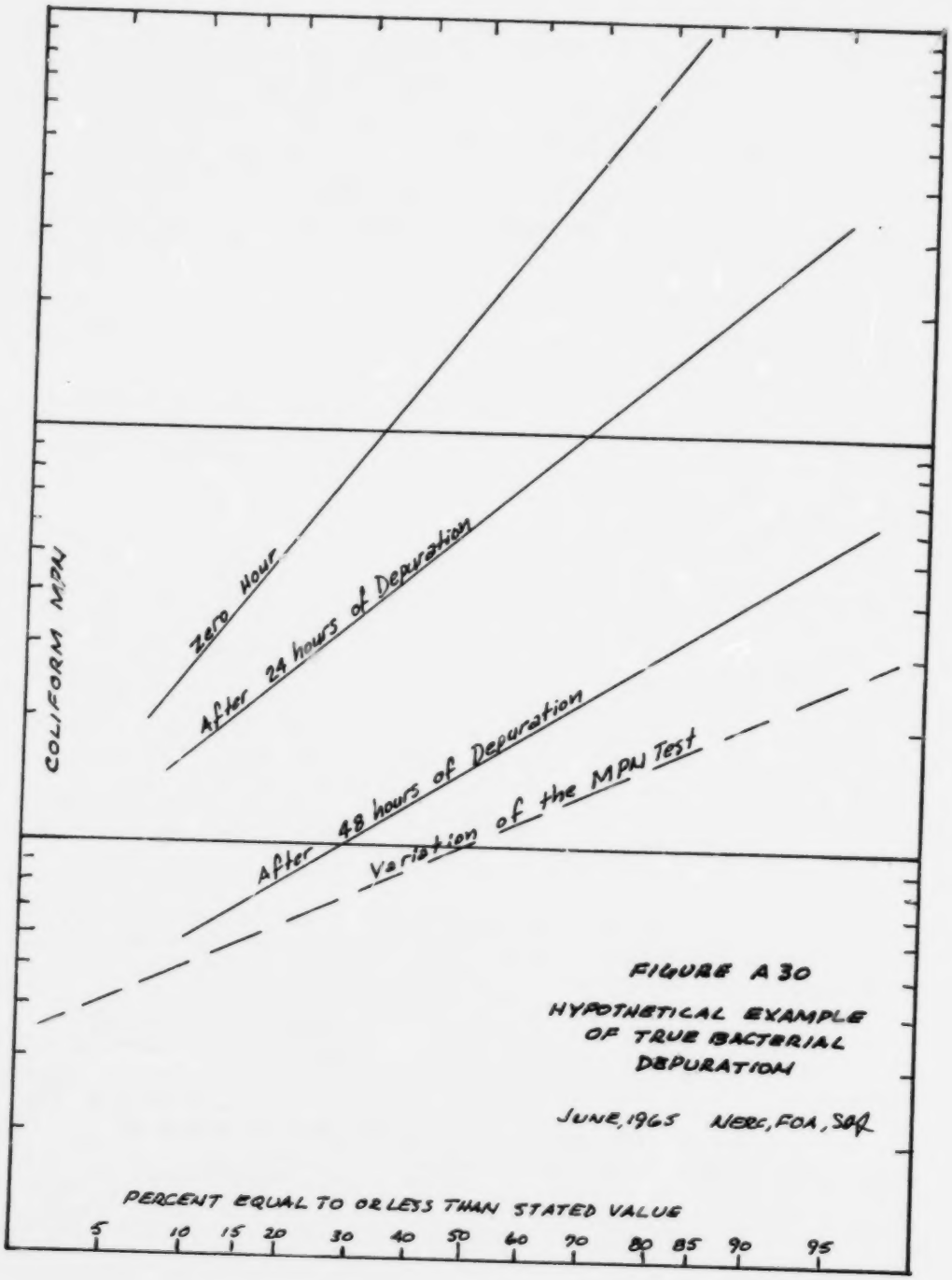

FIGURE A 30

HYPOTHETICAL EXAMPLE OF TRUE BACTERIAL DEPURATION

JUNE, 1965 NERC, FDA, SQR

The method of keeping a record of plant effectiveness is a trend-time plot of the zero hour daily averages and the final outgoing quality daily average. Figure A31 shows a hypothetical plot. A continuous record of this type will ultimately reveal whether the standard is too stringent: that is too stringent to be met consistently given consistent incoming quality and plant operations.

F. Acceptance-Rejection Concepts

Shellfish harvested from approved areas and then depurated should not require as stringent standards as those from restricted areas. The following concepts need not apply in a strict sense to approved area shellfish.

If lots are kept on hand at the plant until a determination can be made of their acceptability, then lots which do not meet standards can be easily placed back into depuration tanks for further treatment.

Before a lot is condemned, however, an investigation into the sample and the source of the lot should be made. The MPN test itself can condemn a sample and therefore the lot, because of the variability inherent in the test. If a running record of the samples from a given harvest area shows consistently good results and the harvester is reputable, these extraneous circumstances alone may militate against rejection of the lot based on one sample. However, if the trend shows consistently poor quality from a given harvest area or harvester then an investigation is warranted.

If the trend lines show consistently poor quality from several harvesters or harvest areas then a tightened inspection program is called for. By this it is meant a) more samples of shellfish, b) sanitary survey of the harvest area c) more samples of the depuration water, before and after treatment, d) an investigation of the activity of the shellfish, and e) checks on laboratory procedures. During these procedures production will still go on and, probably, acceptance of borderline cases will take place.

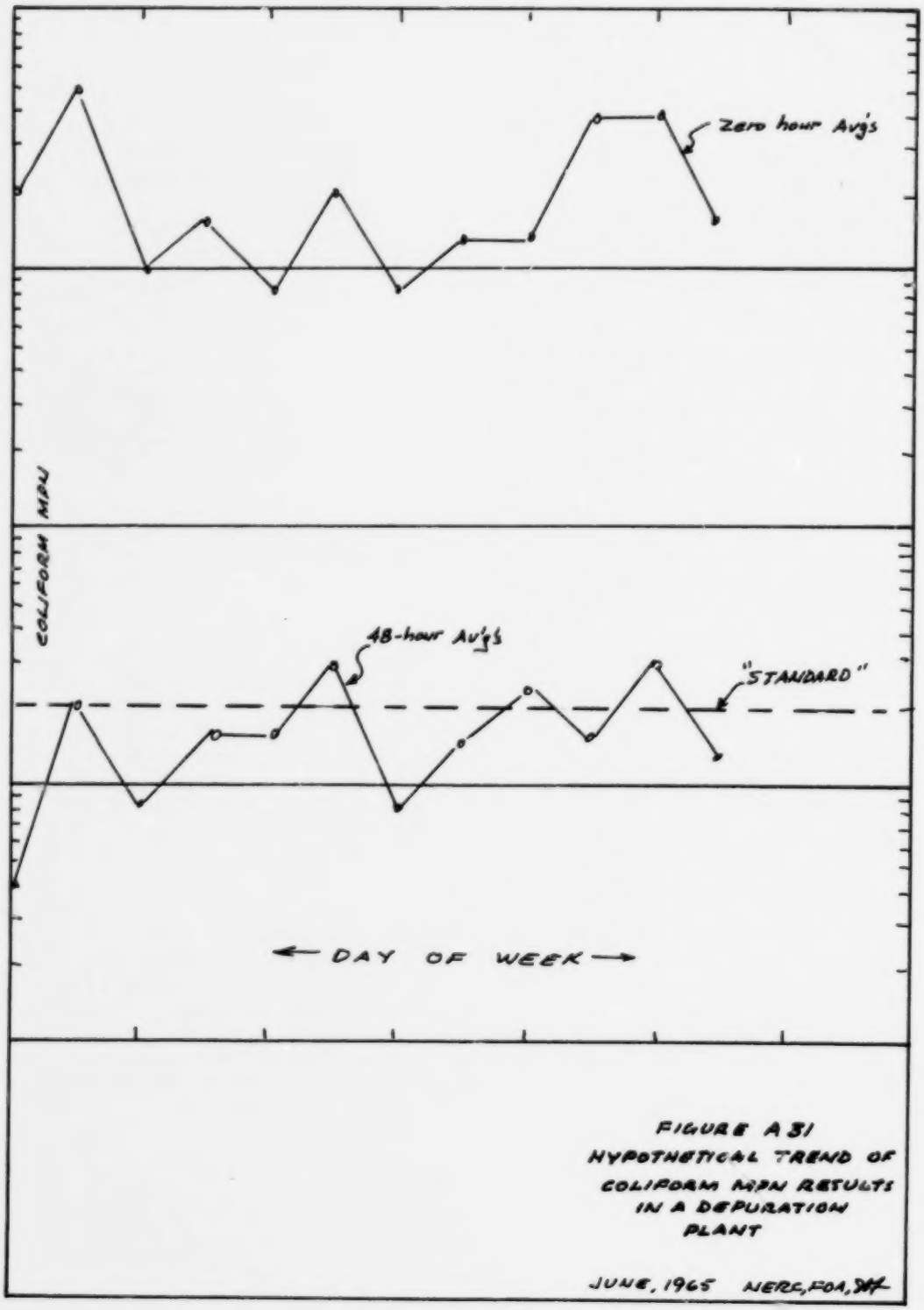

FIGURE A 31
HYPOTHETICAL TREND OF COLIFORM MPN RESULTS IN A DEPURATION PLANT

JUNE, 1965 NERC, FOA, SAF

G. Number of Samples

The guidelines call for a minimum shellfish sampling of at least each harvest area at the various times: as delivered, zero hour, 24 hours, 48 and 72 hours if necessary. In other words not every harvester's lots have to be sampled; only a randomized representative number.

Figure A32 is drawn to show the possible scope of the sources of shellfish for depuration plants. Three different type areas are given and several or many different harvesters from each area. Some harvesters may switch areas from day to day. It is logical to say that a more stringent inspection (sampling) program should be instituted for the restricted harvest area (C) than for the approved harvest area (A).

Under these mixed conditions, shellfish from the different areas must be segregated at the plant during the processes.

In light of the above, a possible sampling program for depuration plants may take the form of:

Number of Harvest Areas	Number of Harvesters	Number of MPN Results per Day*
1	1	Duplicate
1	Several	Alternative 1. Two randomized harvesters' lots in duplicate.
Several	Several	Alternative 2. Three lots (harvesters'). At least one sample per day per area with harvesters randomized.

*For as delivered, zero-hour, 24-, 48-, etc.-hour results.

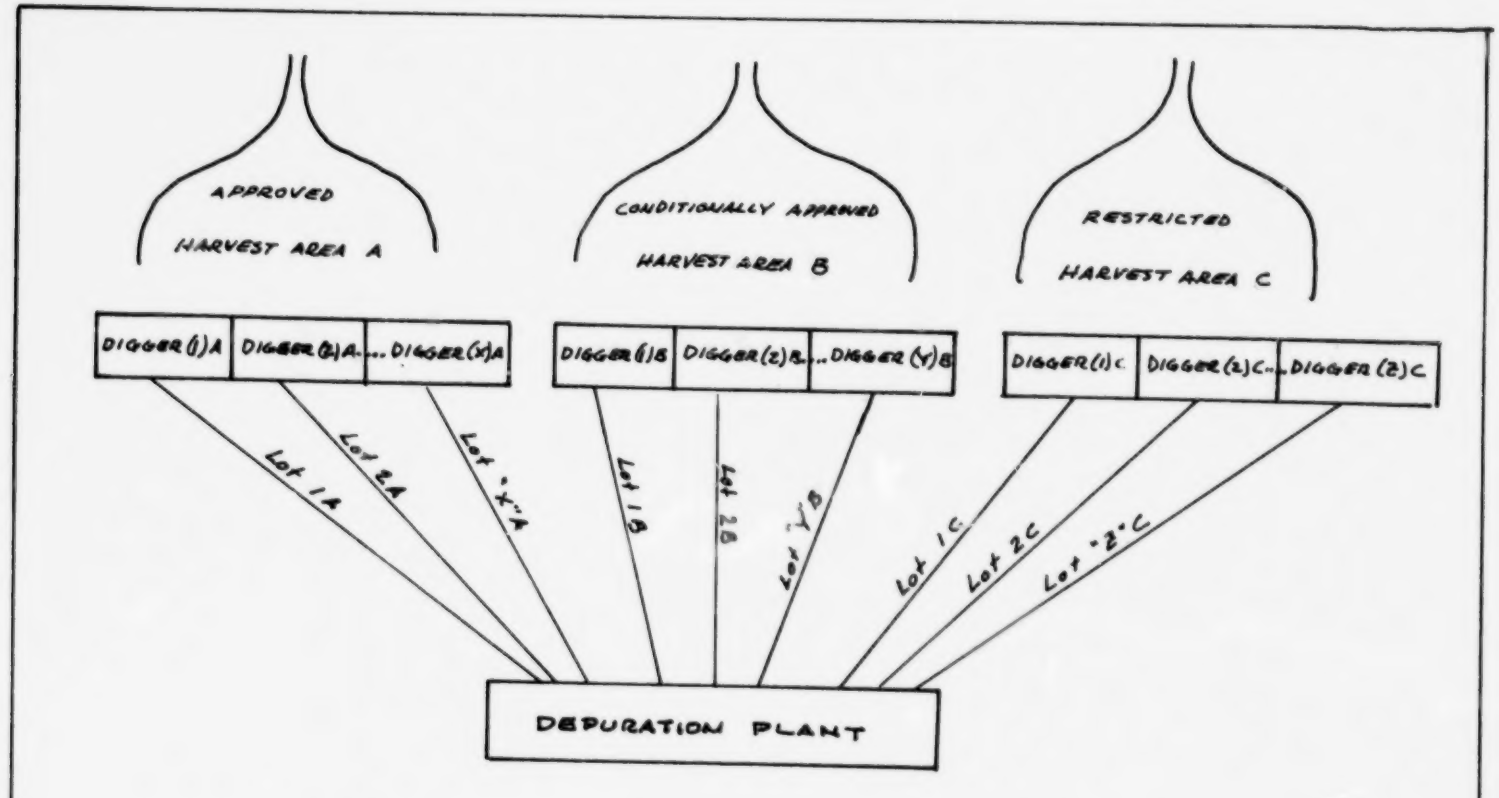

FIGURE A32
TYPICAL DEPURATION PLANT
HARVEST AREA - SHELLFISH
SOURCE RELATIONS

NERC, FOA, SAT June, 1965

BIBLIOGRAPHY

Allen, L. A., et al. 1950. Repeated Re-use of Sea Water as a Medium for the Functioning and Self-Cleansing of Molluscan Shellfish. Jour. Hyg. 48. No. 4, 431-457. (Dec.)

Arcisz, William and Kelly, C. B. 1955. Self-Purification of the Soft Clam, Mya arenaria. PHS Reports, 70(6) June.

Atwood, R. P., et al. 1964. Survival of Viruses in Shellfish. Unpublished Data in CDC, Hepatitis Surveillance, Report #20, Sept. 30, 1964.

Baughman, J. L. 1948. An Annotated Bibliography of Oysters with Pertinent Material on Mussels and other Shellfish and an Appendix on Pollution. Texas A and M Research Found'n, College Station, Texas.

Belding, David L. 1930. The Soft-Shelled Clam Fishery of Massachusetts, Comm. of Mass. Marine Fisheries Series No. 1, Rev. of Nov. 1930.

Bennett, Miriam F. 1954. The Rhythmic Activity of the Quahog, Venus Mercenaria, and its modification by Light. Biol. Bull. 107: 174-191.

Bito, Tomoko and Takase, Akira. 1963. Studies on the Self Decontamination of Living Oysters Using Radioactive Labeled Bacteria. Bull. Inst. Public Health, 12(2):59-64.

*Bodin, E. and Chevrel, F. 1913. Bacterial Purification of Oysters in Filtered Sea Water. C.R.Ac.Sci. Paris. 156:342-5.

Boury, M. and Borde, J. 1964. La Contamination Bacterienne des Coquillage. Sym. Pollut. mar par Microorgan., Monaco, 277-292, April.

Butler, P. A., Lowe, J. I. and Wilson, A. J., Jr. 1963. Uptake and Retention of DDT by Shellfish - Abstract. Bur. Comm. Fish. Biol. Lab. Gulf Breeze, Fla. July 1963.

Canario, M. and Tubman, A. 1965. First Trials with Hard Clams in the Depuration or Purification Process as Evidenced by Coliform Bacteria. Unpublished Report by State of Rhode Island and USPHS.

Carmelia, F. A. 1921. Hypochlorite Process of Oyster Purification. PHS Reports April 22, 1921:876-883.

**Cerruti, A. 1941. Osservazioni ed Esperimenti, - Sulle Cause d. Distruzione della Larve d' Ostrica nei Mar Piccolo e nei Mar Grande d' Taranti. Arch. d' Oceanogr. Limnol. Roma 1:165-201.

Ceurvels, Russel. 1963. Unpublished Data on Soft Clam Depuration From Newburyport, Mass., Plant, Div. of Marine Fisheries.

Chanley, P. E. 1948. Survival of Some Juvenile Bivalves in Water of Low Salinity. Proc. Nat. Shellfish. Assoc. 48.

*Chipman, Walter A. 1947. Seasonal Changes in the Fattening of Oysters. Address before 1947 Oyster Conv., Asbury Park, N. J.

*Coe, W. R. 1947. Nutrition in Filter Feeding Bivalve Molluscs. Abstr. Anat. Rec. 99(4):112.

Collier, Albert, et al. 1953. Effect of Dissolved Organic Substances on Oysters, Fishery Bulletin 84, Vol. 54, USDI.

Crovario, Piero. 1958. Some Observations on the Depuration of Mussels Infected with Poliomyelitis Virus. Presented at Meeting of Ligurian Chapt. of Italian Assoc. of Hygiene, Pietra Italy, Igiene Moderna 51, 1958 (pp.22-32).

Dam L. Van. 1935. On the Utilization of Oxygen by Mya arenaria. Jour. Expti. Biol. 12(1):86-94.

Davis, H. C. and Chanley, P. E. 1955. Spawning and Egg Production of Oysters and Clams, Proc. Nat. Shellfish. Assoc. 46, 50-51.

Dodgeson, R. W. 1928. Report on Mussel Purification. Min. of Agric. and Fish. Ser. II. Vol. 10. No. 1.

*Elsey, C. R. 1936. The Feeding Rate of the Pacific Oyster. Prog. Rept. Pac. Biol. Sta. and Pac. Fish. Exp. Sta. 27:6-7.

Erdman, I. E. and Tennant, A. D. 1956. The Self Cleansing of Soft Clams: Bacteriological and Public Health Aspects. Canad. Jour. Pub. Health. 47:196-202.

*Fabre-Domerque, M. 1912. Bacterial Purification of Oysters Standing in Filtered Artificial Sea Water. Ac. Sci. Paris, 154:393-5.

*Fabre-Domerque, M. 1913. Purifying Oysters from Disease Germs. Naturwiss. Umschau d. Chem. Ztg. 1913(3), Schwez. Wochschr 51, 403-404, Chem. Abstr. 7:4021.

Fair, G. M. and Geyer, J. C. 1959. Water Supply and Waste Water Disposal. John Wiley & Sons, Inc., N. Y.

Feng, Sung Yen. 1963. Activity of the Hard Clam, Mercenaria mercenaria. Talk at Rutgers, the State Univ. of N.J. at NAS Meeting, July.

Feng, Sung Yen. 1965. Talk at NE Research Center, SSB, DEEFP, USPHS, May 6, 1965, Research Conference.

Fester, J. W. and Hunt, D. A. 1964. Bacteriological Criteria for Market Soft Shell Clam. Coop. Study. Maryland-USPHS, Northeast Shellfish San. Res. Center, USPHS, Narragansett, R. I.

*Galtsoff, Paul S. 1928(a). The Effect of Temperature on Mechanical Activity of Gills of Oyster, O. virginica Gm. Jour. Gen. Physiol. 11(4):415-431.

*Galtsoff, Paul S. 1928(b). Experimental Study of the Function of the Oyster Gills and Its Bearing on the Problems of Oyster Culture and Sanitary Control in the Oyster In istry. Bull. U.S. Bur. Fish. XLIV 1-39.

*Galtsoff Paul. 1932. The Life in the Ocean from a Biochemical Point of View. J. Wash. Acad. Sci. 22(9):246-257.

Galtsoff, P. S. 1946. Reaction of Oysters to Chlorination. U.S. Fish and Wild. Serv. Res. Rpt. 11, 1-28.

*Galtsoff, P. S. and Whipple, D. 1931. Oxygen Consumption of Normal Green Oysters. Bull. U.S. Bur. Fish. XLVI 489-508.

Galtsoff, P. S. 1964. The American Oyster, Crassostrea virginica. Fishery Bulletin, Vol. 64, USDI.

Goggins, P. L., et al. 1964. Soft Clam Depuration Studies. Maine Dept. of Sea and Shore Fisheries, Augusta, Maine.

Hammen, Carl S., Hanlon, D. P. and Lum, S. C. 1962. Oxidative Metabolism of Lingula. Comp. Bioch. Physiol. 5:185-191.

Hedstrom, C. and Lycke, E. 1963. An Experimental Study on Oysters as Virus Carriers. Am. Journ. of Hyg. 79:134-142.

***Herdman, W. A. and Boyce. 1899. Oysters and Disease. Lancashire Sea Fisheries Memoir I, Publ. by George Phillips and Son.

Hope, J. A. and Wiley, C. W. 1961. Coliform E.C. Gas Positive Relationships in Shellstock, Sea Water and Bottom Sediments from Areas of Varying Bacterial Quality. Proc. Shellfish San. Workshop, HEW, PHS, 1961.

*Hopkins, A. E. 1931. Temperature and Shell Movements in Oysters. Bull. U.S. Bur. Fish. XLVII, 1-14.

*Hopkins, A. E. 1933. Experiments on the Feeding Behavior of the Oyster (O. gigas). Jour. Exp. Zoo. 64(3)469-494.

Hopkins, A. E. 1936. Adaption of the Feeding Mechanism of the Oyster (O. gigas) to Changes in Salinity. Bull. U.S. Bur. Fish. XLVIII, 345-364.

**Ingle, R. M. and Dawson, C. E. 1950. Variation in Salinity and Its Relation to the Florida Oyster: Salinity in Apalachicola Bay. Proc. Gulf. Carib. Fish. Inst. 1950:3-42.

***Johnstone, J. 1914. The Methods of Cleaning Living Mussels from Ingested Sewage Bacteria. Rep. for 1914 on the Lancashire Sea Fisheries Lab. No. XXIII.

Kehr, R. W., et al. 1941. A Report on the Public Health Aspects of Clamming in Raritan Bay. USPHS, DHEW.

Kelly, C. B. 1956. Bacteriological Examination as an Indicator of Sanitary Quality of Market Shellfish. Proc. 1956 Shellfish Sanitation Workshop. 85-94.

Kelly, C. B., et al. 1960. Bacterial Accumulation by the Oyster, Crassostrea virginica, on the Gulf Coast. RATSEC, Tech. Rept. F60-4, USPHS, DHEW.

Kelly, C. B. 1961(a). Purification of Oysters. 1961 Shellfish Sanitation Research Conference, Purdy, Washington.

Kelly, C. B. 1961(b). Disinfection of Sea Water by UV Radiation. Am. J. Pub. Health. 51(11).

***Klein. 1905. Experiments, etc., on the Vitality of the Bacillus of Typhoid Fever and of Sewage Microbes in Oysters and Other Shellfish, on behalf of the Worshipful Company of Fishmongers.

Korringa, P. 1952. Recent Advances in Oyster Biology. Quart. Rev. of Biol. Vol. 27. 266-308, 339-365.

*Krumweide, Charles, et al. 1926. Effect of Storage and Changing of Sea Water on Contaminated Oysters. Am. Jour. Pub. Health. 16(3):263-268.

*Krumweide, C., et al. 1928. The Purification of Contaminated Oysters in Natural Waters. Am. Jour. Pub. Health and Nations Health, 18(1):48-52.

Liu, Oscar C., et al. 1965. Accumulation and Elimination of Poliovirus by Northern Quahogs with Corroborative Data using E. coli. Synoptic Report, September 15, 1965, Northeast Shellfish Sanitation Research Center, USPHS, DHEW.

Loosanoff, V. L. 1952. Behavior of Oysters in Water of Low Salinities. Conven. Address. National Shellf. Assoc. Atlantic City.

Loosanoff, V. L. 1958. Some aspects of behavior of Oysters at Different Temperatures. Biol. Bull. 114(1):57-70.

Loosanoff, V. L. 1961. Effects of Turbidity on Some Larval and Adult Bivalves. Proc. Gulf and Carib. Fish. Inst. 14th Session. Nov. 1961: 80-95.

*Loosanoff, V. L. and Engle, J. B. 1942. Effects of Different Concentrations of Plankton Forms upon Shell Movements, Rate of Water Pumping and Feeding and Fattening of Oysters. Anat. Rec. 84:536-537.

*Loosanoff, V. L. and Engle. 1947. Effect of Different Concentrations of Microorganisms on Feeding of Oysters. Fishery Bull. 42, Fish. Bull. U.S. Fish and Wild. Ser. 51:31-57.

**Loosanoff, V. L. and Nomejko, Charles A. 1946. Feeding of Oysters in Relation to Tidal Stages and to Periods of Light and Darkness. Biol. Bull. Woods Hole 90:244-264.

*Loosanoff, V. L. and Tommers, F. D. 1947. Effect of Low pH upon Rate of Water Pumping of Oysters (O. virginica). Anat. Rec. 99(4):112-113.

Marston, Alice T. 1930. The Effects of Temperature Upon the Ingestion of Bacteria by the Clam (Mya arenaria). Trans. Amer. Fish. Soc. 60:269.

Massachusetts, Comm. of. 1947. Report of the Special Commission Established to Make an Investigation and Study Relative to Edible Shellfish and Shellfish Chlorinating Plants. H.R. 1770, December.

Matthiesson, George C. (1960) Observations on the Ecology of the Sott Clam, Mya arenaria, in a Salt Pond. Woods Hole Oc. Inst., Coll. Repr., Contr. No. 1056.

McFarren, E. F., et al. 1961. Occurrence of Copper and Zinc in Shellfish. Proc. Shellfish San. Workshop.

**Medcof, J. C. 1944. How Relaying and Transferring at Different Seasons Affects the Fatness of Oysters. Prog. Rpt. Atl. Biol. Sta. 35:11-14.

Messer, Richard and Reece, G. M. 1937. Progress in Oyster Conditioning. P. H. Reports 1451-1460.

Metcalf, T. G. and Stiles, W. C. 1965. The Accumulation of Enteric Viruses by the Oyster, Crassostrea virginica. J. Inf. Dis. Feb., 1965.

Mitchell, J. R., et al (1966). Accumulation and Elimination of Poliovirus by the Eastern Oyster. USPHS. Gulf Coast Research Center.

*Mitchell, P. H. 1914. The oxygen Requirements of Shellfish. Bull. U.S. Bur. Fish. 32:207-222.

Nelson, J. Richards. 1934. Controlled Water Storage and Conditioning of Oysters for Market. N. J. Agric. Coll. Expt. Sta. Cir. 322.1.8.

*Nelson, Thurlow C. 1923. On the Feeding Habits of the Oyster. Proc. Soc. Expt. Biol. Med. 21(2):90-91.

*Nelson, Thurlow C. 1927. On the Relation of Spawning of American Oyster to Temperature. Anat. Rec. Suppl. 144.

*Nelson, Thurlow C. 1928. On the Distribution of Critical Temperatures for Spawning and Ciliary Activity in Bivalve Mollusca. Science 67:220-221.

O'Donovan, D. C. 1965. Treatment with Ozone. JAWWA. 57, #9. 1167-1192 (Sept.).

**Pedersen, E. 1947. Østersens Respirasjon. Undersøkelser utført ved Statens Utklenkningsanstalt Flødevigen. Fiskeridir. Skr. Havundersøk. 8:1-51.

Petrilli, Fernando Luigi. 1938. Ricerche sull' autodepurazione dei mitili. L'Igiene Moderna. 30:309.

Phillips, G. Briggs, and Hanel, Everett, Jr. 1960. Use of Ultraviolet Radiation in Microbiological Laboratories. U.S. Army Chem. Corp., Fort Detrik, Tech. Rpt. BL.28.

Pierce, Madelane. 1950. Selected Invertebrate Types: Frank A. Brown, Editor. John Wiley & Sons, Inc. 324-334.

Presnell, M. W. and Cummins, J. M. 1965. Effectiveness of Ultraviolet Radiation Units in the Bactericidal Treatment of Sea Water. Unpublished Report. USPHS. Gulf Coast Research Center.

Presnell, M. W. and Kelly, C. B. 1961. Bacteriological Studies of Commercial Shellfish Operations on the Gulf Coast. Robt. A. Taft San. Eng. Cntr. Techn. Rept. No. F61-9.

Rice, T. R. and Smith, R. J. 1958. Filtering Rates of the Hard Clam (V. mercenaria) determined with Radioactive Plankton, Fish. Bull. 58(129).

Shaw, William N. (1963?) Seasonal Gonadal Cycle of the Male Soft Shell Clam, Mya arenaria, in Maryland. U.S.F.W.L. Serv., Spec. Scien. Rpt. No. 508.

Shuster, C. N., 1952. Preliminary Survey: Soft Shell Clam, Mya arenaria (L), Populations in the Rivers and Bays in the Region of Sandy Hook, New Jersey. Dept. Zool. Rutgers, State Univ. of New Jersey.

Stauber, L. A. 1961. Immunity in Invertebrates, with Special Reference to the Oyster. Proc. Nat. Shellfish Assoc. 50.

Summer, W., Dr. 1962. Ultraviolet and Infrared Engineering. Interscience Publishers, Inc., N. Y.

*Violle, H. 1929. The Sterilization of Sea Water by Ozone. Rev. Hyg. Med. Prev. 51:42-46.

Wells, H. W. 1961. The Fauna of Oyster Beds with Special Reference to the Salinity Factor. Ecol. Mono. 31:239-266 (Summer).

Wells, W. F. 1916. Artificial Purification of Oysters. Pub. Health Reports. 31:1848-1852.

*Wells, W. F. 1923. Purification Plant for Oysters from Polluted Water. Eng. News Rec. 90:1047.

*Wells, W. F. 1929. Chlorination as a factor of Safety in Shellfish Production. Am. Jour. Pub. Health. 19:72-79.

Wood, P. C. 1961a. The Principles of Water Sterilization by Ultraviolet Light and their Application in Purification of Oysters. Brit. Min. of Agric. and Food. Fish. Invest. Ser. II, Vol. 23, No. 6.

Wood, P. C. 1961b. The Production of Clean Shellfish. Presented at a meeting of the Royal Society of Health, Chelmsford, January 25.

Parts I and II. National Shellfish Sanitation Program, Manual of Operations, USDHEW.

NOTES:

1. References marked * -- Information derived from material given in Baughman (1948) - Annotated Bibliography.

2. References marked ** -- Information derived from material given in Korringa (1952) - Quarterly Jour. of Biology.

3. References marked *** -- Information derived from material given in Dodgeson (1928) - Report on Mussel Purification.

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